NUCLEIC ACID RATIOS AS AN INDEX OF GROWTH AND NUTRITIONAL ECOLOGY IN PACIFIC COD (*GADUS MACROCEPHALUS*), WALLEYE POLLOCK (*THERAGRA CHALCOGRAMMA*), AND PACIFIC HERRING (*CLUPEA PALLASII*)

A

DISSERTATION

Presented to the Faculty

of The University of Alaska Fairbanks

in Partial Fulfillment of the Requirements

for the Degree of

DOCTOR OF PHILOSOPHY

By

Ashwin Sreenivasan, B.S., M.S.

Fairbanks, Alaska

December 2011

ProQuest LLC
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106-1346

NUCLEIC ACID RATIOS AS AN INDEX OF GROWTH AND NUTRITIONAL ECOLOGY IN PACIFIC COD (*GADUS MACROCEPHALUS*), WALLEYE POLLOCK (*THERAGRA CHALCOGRAMMA*), AND PACIFIC HERRING (*CLUPEA PALLASII*)

By

Ashwin Sreenivasan

RECOMMENDED:

Dr. William Smoker, Advisory Committee Chair

Dr. Milo Adkison, Chair, Graduate Program in Fisheries Division

APPROVED:

Dr. Michael Castellini, Dean, School of Fisheries and Ocean Sciences

Dr. Lawrence Duffy, Interim Dean of the Graduate School

Dec 6, 2011

Date

Abstract

Pacific cod (*Gadus macrocephalus*), walleye pollock (*Theragra chalcogramma*), and Pacific herring (*Clupea pallasii*) are among the most ecologically and commercially important species in the North Pacific Ocean. In spite of their importance, little is known about larval and juvenile growth strategies in these fish. Since larval and juvenile fish growth may determine future growth, possibly affecting recruitment success, assessments of growth strategies might improve predictive growth models

Nucleic acid ratios (RNA/DNA) can have applications as a sensitive growth index in larval and juvenile Pacific cod, walleye pollock, and Pacific herring, and can potentially be used to determine growth responses and energetic assessments at the cellular level. Determining physiological growth responses in these fish after exposure to different temperatures and nutritional states can help in understanding growth strategies and condition. Nucleic acid ratios from white muscle of juvenile Pacific herring and whole-body Pacific cod and walleye pollock larvae were used as a cellular growth index to provide energetic assessments in these species. Growth responses were studied in these fish across a range of temperatures and nutritional states.

Growth was compared between fed, starved/fed and terminally starved Pacific herring cultured at 6.5°C, 8.5°C, and 12.5°C. Relative to fed controls, starved/fed fish showed similar RNA/DNA ratios and soluble protein concentration, but reduced mass. Nucleic acid ratios in starved/fed fish during the starvation phase, and in terminally starved fish, indicated incipient terminal starvation. Also, a seasonal variation of

RNA/DNA, protein concentrations and total body lipid concentrations was seen in fed fish, reflecting changes in resource allocation.

Early growth was compared in yolk-sac Pacific cod and walleye pollock larvae cultured at 5°C and 8°C, and in yolk-sac Pacific cod larvae cultured in two nutritional states (fed and starved). Growth responses in Pacific cod and walleye pollock larvae were affected by small differences in temperature. Exposure to the lower temperature resulted in higher RNA/DNA in both Pacific cod and walleye pollock larvae. Based on nucleic acid patterns with larval development, it was possible to identify distinct growth stanzas in Pacific cod larvae.

Table of Contents

List of Figures

List of Tables

Acknowledgements

Several people have assisted me through the duration of my research in numerous ways, and I would like to express my deep gratitude for their support and encouragement. I firstly thank all my committee members for their insight in integrating numerous physiological concepts within a fisheries framework. My advisor, Dr. Bill Smoker (UAF), and Dr. Jeep Rice (NOAA) have been especially helpful in this regard, as also with their editorial reviews and comments. I am also very grateful to Dr. Ron Heintz (NOAA) for being extremely generous with his time, as well as for the numerous conceptual discussions and direction provided by him over the course of my research. I greatly appreciate all the help I received from Elaine Caldarone (NOAA), who was instrumental in helping me adapt her nucleic acid ratio protocol to my research. Numerous sources have supported me financially over the course of this research, to which I am grateful, including the Rasmuson Fisheries Research Center, the University of Alaska Fairbanks, and the NOAA Alaska Fisheries Science Center. I am also thankful to NOAA for supporting my research by providing me with all facilities required for the completion of this research project. I was responsible for all biochemical analyses of tissue nucleic acids and protein, statistical methods of analyses and modeling, and manuscript preparation. Ronald Heintz and Johanna Vollenweider were responsible for study designs and coordination as well as lipid analyses, and also assisted with statistical analyses and manuscript preparation. Paul Hershberger contributed to the study design and supervised culturing and sampling of Pacific herring. Jacob Gregg was responsible for culturing and sampling of Pacific herring. Thomas Hurst and Benjamin Laurel were

responsible for the Pacific cod and walleye pollock study design, and also supervised culturing and sampling of Pacific cod and walleye pollock. Stanley Rice was responsible for overall study design. I had no involvement in collection, care, or handling of live fish and all tissue samples utilized in chapter 1 and chapter 2 were opportunistically supplied to me from experiments being carried out by federal scientists from NOAA Auke Bay Laboratories Ted Stevens Marine Research Institute. All animal collections, care, and handling were the responsibility of NOAA scientists as part of their experiments, and complied with NOAA internal standards (appendix). The original experimental designs were not altered in any way while sampling fish for my studies. All samples utilized in chapter 1 were opportunistic, and experiments involving live vertebrate test animals were performed under an approved USGS-Western Fisheries Research Center Institutional Animal Care and Use Committee protocol (Protocol#2008-14). All samples utilized in chapter 2 were opportunistic, and provided to me by agreement with NOAA (appendix).

General Introduction

In Alaskan waters, Pacific cod (*Gadus macrocephalus*), walleye pollock (*Theragra chalcogramma*), and Pacific herring (*Clupea pallasii*) are among the most ecologically and commercially important fish species. Pacific cod and walleye pollock are among the most numerous (Mecklenburg et al. 2002) and commercially important species in the North Pacific Ocean (Jewett 1978, Grant et al. 1987, Stepanenko 1995, Beamish et al. 2004, Bacheler et al. 2010). Pacific herring, in addition to being an important economic resource (Foy and Paul 1999), are an important prey species for fish, birds, and marine mammals in the North Pacific Ocean (Womble et al. 2005, Suryan et al. 2006).

Pacific cod

Pacific cod have a distribution ranging from southern California north to St. Lawrence Island in the Bering Sea, along the Aleutian Islands, the Gulf of Anadyr and along the Kurile Islands, the Okhotsk Sea, the Yellow Sea, and the Sea of Japan (Ketchen 1961, Bakkala 1984, Bakkala et al. 1984), and are an important upper-trophic level species in subarctic ecosystems (Sakurai and Hattori 1996). Pacific cod are particularly abundant in the Bering Sea (Klovach et al. 1995), the Aleutian Islands, and the Gulf of Alaska (Shimada and Kimura 1994), and are an important component of the food web in both the North Pacific Ocean and the Bering Sea (Hurst et al. 2010).

Mature Pacific cod are normally found in inshore and offshore areas extending out to the continental shelf (Abookire et al. 2001), while juvenile Pacific cod inhabit relatively shallow waters (Bakkala 1984) as observed off Kodiak Island, Alaska (Mueter and Norcross 1999). While normally benthic, adult Pacific cod can be pelagic. Pacific

cod can range from near the surface to as deep as 875 m (Mecklenburg et al. 2002), but are usually found at depths less than 350 m. Pacific cod normally inhabit water temperatures ranging from 0°C-10°C (Ketchen 1961, Shimada and Kimura 1994). However, Pacific cod off the Asian coast inhabit cooler temperatures (0°C-4°C) than Pacific cod off the North American coast (Ketchen 1961).

Pacific cod can be an important predator on other marine species in the North Pacific Ocean ecosystem (Paul et al. 1990). Pacific cod can prey on a wide range of species, including walleye pollock, crab (*Chionoecetes* spp.), shrimp (primarily *Pandalus borealis* and *P. goniurus*), flatfish, Pacific herring, and young Pacific cod (Jewett 1978, Bakkala 1984, Zhang 1984, Albers and Anderson 1985, Rovnina et al. 1997).

Pacific cod normally reach sexual maturity at age 4+, with high natural mortality rates observed after age 7+ (Stepanenko 1995). The maximum age of cod can be 13+ years (Klovach et al. 1995). Pacific cod normally spawn in the late winter/early spring period, from December to April (Ketchen 1961, Bakkala 1984, Shimada and Kimura 1994). Pacific cod are highly fecund (Rovnina et al. 1997), and spawn once during the spawning cycle, laying demersal eggs over rocky substrates between 20-200 m depth (Hurst et al. 2009). After hatching, yolk-sac larvae exhibit a strong surface orientation (Hurst et al. 2009), rapidly moving to the upper water column.

Walleye pollock

Walleye pollock are distributed from the Bering Sea and the Gulf of Alaska (GOA) to Japan in the northeast and northern Californian waters (Mecklenburg et al. 2002, Bacheler et al. 2010). Walleye pollock are particularly abundant in the Bering Sea

as well as the Sea of Okhotsk (Bailey et al. 1997, Ciannelli et al. 2007). They are an important component of the food web in the Bering Sea (Napp et al. 2000, Wespestad et al. 2000), both as predators (Brodeur et al. 2000) and as prey for numerous species of fish, birds, and marine mammals (Lang et al. 2000, Wespestad et al. 2000, Sinclair et al. 2008).

Walleye pollock are a subarctic species (Mueter and Litzow 2008), inhabiting water temperatures ranging from 1°C-10°C over a range of habitats (Bacheler et al. 2010). While normally found in waters over outer shelf and slope regions, they can also inhabit eelgrass beds, coastal embayments, estuarine areas, and open ocean waters (Bailey et al. 1997). Walleye pollock are demersal, normally occupying depths of 30-300 m (Mecklenburg et al. 2002), but can also be found near surface waters.

Walleye pollock prey on a variety of marine organisms, including copepods, epibenthic species, as well as other fish (Bailey et al. 1997). Adult fish can be highly cannibalistic (Livingston 1993), preying heavily on juveniles. Juvenile walleye pollock also have a highly varied diet, feeding on zooplankton including copepods, euphausiids, pteropods, as well as fish and epibenthic crustaceans (Theilacker et al. 1996, Brodeur et al. 2000, Ciannelli et al. 2004).

Walleye pollock reach sexual maturity between ages 3-4+ (Bacheler et al. 2010). Spawning normally begins in the Bering Sea in January, but the onset of spawning could be spread out over a period of 8 months (Hinckley 1987) depending on location. Walleye pollock spawn mature eggs in successive batches. Spawning normally takes place in outer regions of the continental shelf, including valleys and canyons, with eggs found at

depths of 100-400 m (Bailey et al. 1997). After hatching, larvae gradually move to the upper water column (Bailey et al. 1997, Hurst et al. 2009)

Pacific herring

Pacific herring have a distribution ranging from California waters north through Alaska and over to the northeastern Pacific Ocean through Asia (Haegele and Schweigert 1985, Mecklenburg et al. 2002). Pacific herring are a schooling species (Marty et al. 2010), and are important in the food web of the North Pacific Ocean both as secondary consumers and as prey for a variety of fish, birds, and marine mammals (Tanasichuk et al. 1991, Womble et al. 2005, Suryan et al. 2006).

Mature Pacific herring are normally found nearshore, inhabiting continental shelf zones (Carlson 1980, Haegele and Schweigert 1985, Tanasichuk et al. 1993), while larvae and juveniles inhabit inshore nursery areas (Hay 1985, McGurk et al. 1993, Norcross et al. 2001). Pacific herring can range from the surface to depths of up to 250 m, but are normally found at depths less than 150 m (Mecklenburg et al. 2002).

Pacific herring are planktivores and feed near the sea surface (Mecklenburg 2002). The diet of juvenile Pacific herring varies seasonally (Foy and Norcross 1999), and can comprise a wide variety of copepods, including *Pseudocalanus* spp. and *Metridia* spp., euphausiids, amphipods, polychaetes, and even fish eggs (Foy and Paul 1999, Norcross et al. 2001).

Pacific herring normally reach sexual maturity between ages 3-5+ (Hay 1985), and can live up to 12+ years (Marty et al. 2010). While spawning times can vary between populations (Haegele and Schweigert 1985), most Pacific herring spawn

between March and April (Hay 1985), normally within a 3-6 week period (Haegele and Schweigert 1985). Pacific herring spawn in tidal and subtidal areas (Norcross et al. 1996) in sheltered inlets and bays, laying eggs on kelp and marine vegetation (Alderdice and Velsen 1971, Haegele and Schweigert 1985). Over the spawning period, larger and older Pacific herring tend to spawn earlier than smaller fish (Hay 1985). After hatching, larval fish can be carried by local currents to inshore nursery areas (McGurk et al. 1993).

Most of the commercial fishery for Pacific cod and walleye pollock in Alaskan waters occurs in the Bering Sea/Aleutian Islands area and the GOA (Hiatt et al. 2010). The walleye pollock fishery is considered among the largest in the world, and has traditionally accounted for a large portion of biomass in the groundfish fishery (~56% in 2009). The Pacific cod fishery is the second largest following walleye pollock, accounting for ~15% of the biomass in the 2009 groundfish fishery. The walleye pollock fishery in Alaskan waters for the year 2009 was valued at ~$308M (ex-vessel value), while the Pacific cod fishery was valued at $111M. The Pacific herring fishery in Alaskan waters for the year 2009 was valued at ~$29M (ex-vessel value; Hiatt et al. 2010)

However, with the exception of a few studies (Alderdice and Forrester 1971, Sogard and Olla 1996, Theilacker et al. 1996, Foy and Paul 1999, Foy and Norcross 1999, Norcross et al. 2001, Laurel et al. 2008, Hurst et al. 2009, Hurst et al. 2010, Laurel et al. 2010, Laurel et al. 2011), there is relatively little information on larval and juvenile growth parameters in these species. As larval growth patterns may determine future growth (Jonsson et al. 2005), possibly affecting recruitment success, estimating larval and

juvenile growth strategies can be important. Studies so far have highlighted the complexities in larval behavior and physiology in the early post-hatch period. Diel migratory behavior in larval Pacific cod can be determined by development stage, temperature, and photoperiod (Hurst et al. 2009), and coincides with increased growth after yolk absorption, together with heightened responsiveness to prey (Colton and Hurst 2010). Growth in turn can be affected by interacting factors, including prey availability, temperature, and development stage (Laurel et al. 2011).

Studies on related species, namely Atlantic cod (*Gadus morhua*) and Atlantic herring (*Clupea harengus*; Houde 1989, Pelletier et al. 1993*a, b*, 1994, 1995, Claireaux et al. 1995*a, b*, 2000, Guderley et al. 1996, 2003, Pepin et al. 1997, Couture et al. 1998, Martinez et al. 2002, 2003, Engelhard and Heino 2005, 2006) have highlighted many physiological and behavioral effects of changing temperature and food availability, and could suggest potential responses in Pacific cod, walleye pollock, and Pacific herring.

Atlantic cod are very sensitive to changes in temperature and dissolved oxygen. Fish in a water column of heterogenous temperatures actively sought out the zone at their acclimation temperature (Claireaux et al. 1995*a*, DeBlois and Rose 1995). The physiological effects of increased temperature in Atlantic cod include large increases in metabolic rate after feeding, possibly affecting availability of energy for other purposes (Claireaux et al. 1995*a*). Heightened temperature also affected early development in Atlantic cod, increasing size of larvae at hatch (Pepin et al. 1997). Atlantic cod are also among the earliest migrants from hypoxic zones (Boutilier 1998). Even within a species, the effects of temperature change can vary between populations (Mieszkowska et al.

2009). Recruitment in 21 stocks of Atlantic cod was affected by temperature, but the effects were dependent on the original thermal habitat of the stock (Mantzouni and MacKenzie 2010). Warm habitat stocks had greater recruitment during colder spawning seasons, while the opposite pattern was observed for cold habitat stocks.

Atlantic herring energy allocation patterns can be affected by changes in temperature and condition. Atlantic herring can skip second spawning seasons (and possibly more) due to climatic and physiological factors, primarily temperature, condition, and growth (Engelhard and Heino 2005, 2006). The effects of environmental change on Atlantic cod and Atlantic herring could suggest similar sensitivities to environmental conditions in Pacific cod, Pacific herring, and walleye pollock.

The growth responses of Pacific cod, walleye pollock, and Pacific herring can be of concern in the North Pacific Ocean, where climate variability or regime shifts have been documented on multi-decadal scales (Francis et al. 1998, Benson and Trites 2002). These regime shifts have affected resident species health and composition (Brodeur and Ware 1992, Anderson and Piatt 1999). Regime shifts have been documented in 1977 (Venrick et al. 1987, Brodeur and Ware 1992, Beamish and Bouillon 1993, Francis and Hare 1994, Benson and Trites 2002, Mantua and Hare 2002, Overland and Stabeno 2004), as well as 1925 and 1947 (Trenberth 1990, Tanimoto et al. 1993, Mantua et al. 1997, Minobe 1997). Possible shifts are thought to have occurred in 1989 (Overland et al. 1999) and 1998 (McFarlane et al. 2000) as well. Regime shifts can affect ocean water temperature, with alternating cold and warm regimes (Freeland 1990, Overland and Stabeno 2004).

Biological resources are affected in conjunction with these climatic variations (Mantua and Hare 2002) in both the Bering Sea (Overland and Stabeno 2004) and the GOA (Anderson et al. 1997). Changes during the shift to a warm-water regime in 1977 included variations in zooplankton biomass and chlorophyll concentrations (Brodeur and Ware 1992, Overland and Stabeno 2004), increased groundfish recruitment and Pacific salmon catches in Alaska (Hollowed and Wooster 1992, Francis and Hare 1994, Mueter and Norcross 1999), as well as fluctuations in abundance of forage species, such as pandalid shrimp and capelin (*Mallotus villosus*; Anderson and Piatt 1999).

Temperature and Nutrition: Effects at the Ecosystem and Cellular Level

Temperature can exert a variety of effects on marine organisms (Portner et al. 2007), ranging from the ecosystem level (Root et al. 2003, Hiddink and Hofstede 2008) to the molecular level (Portner et al. 2007). A few direct effects of steadily heightened temperature in the marine ecosystem can include increased thermal stratification, decreased sea ice, changes to nutrient upwelling patterns and ocean acidification (Thuiller 2007, Hoegh-Guldberg and Bruno 2010). Steadily heightened temperature could also have a few broad-scale effects across taxa. These include a shifting of species towards higher latitudes (comprising poleward movement) where cooler conditions prevail (Parmesan and Yohe 2003, Root et al. 2003, Perry et al. 2005, Hiddink and Hofstede 2008, Cheung et al. 2009, Forister et al. 2010), a shift in the phenology or timing of critical life-cycle events (Walther 2002, Root et al. 2003, Bradshaw and Holzapfel 2010) normally triggered by specific temperature cues, and possibly an overall reduction of body size (Atkinson 1994, Root et al. 2003, Daufresne et al. 2009) in aquatic ectotherms.

Changes in temperature and nutrition can also have effects at the cellular level (Root et al. 2003, Portner et al. 2007), affecting growth and protein synthesis (Treberg et al. 2005, Fraser and Rogers 2007, Lewis and Driedzic 2007). Temperature can affect growth by influencing the primary endocrine factors regulating growth, such as the growth hormone (GH)-insulin-like growth factor (IGF-1) axis. This axis, a primary endocrine factor controlling growth in fish, can be affected by increasing temperature (Gabillard et al. 2003*b*), with heightened GH secretion affecting larval fish growth. Rainbow trout (*Oncorhynchus mykiss*) larvae and juveniles (Gabillard et al. 2003*a*, Li and Leatherland 2008) had heightened GH secretion with elevated temperature, consequently increasing IGF-1 production. Embryo rearing temperature and diet also affected tissue IGF-1 levels, effecting future growth changes.

Insulin-like growth factor-2 (IGF-2), potentially influenced by temperature and diet, can also determine larval fish growth (Duan 1998). Gene expression for IGF-1 and IGF-2 in rainbow trout was affected by nutritional status (Li and Leatherland 2008), while gene expression of IGF receptors (IGF-RIa and IGF-RIb) in larval and juvenile rainbow trout was influenced by temperature (Gabillard et al. 2003*a*, Li and Leatherland 2008). While IGF-1 in juvenile rainbow trout was more sensitive to temperature, plasma IGF-2 was more sensitive to nutritional status (Gabillard et al. 2003*a*), and may also have a greater regulatory role than IGF-1 during the larval-juvenile transition period (Duan 1998, Gabillard et al. 2006). Temperature can affect synthesis of other factors involved in larval fish growth, including IGF receptors and IGF-binding proteins (Picha et al.

2008), determining the growth effects of IGF (Duan et al. 1999, Bauchat et al. 2001, Shimizu et al. 2003, Shimizu et al. 2005).

Temperature can also affect physiological adaptations in fish (Farrell 2009). One example of this can be aerobic scope, the difference between the minimum and maximum rates of oxygen consumption in an organism. Aerobic scope peaks at an optimal temperature, and then decreases (Portner and Knust 2007, Farrell 2009), due to continued exponential increase in standard metabolic rate with heightened temperature, but not in maximum metabolic rate. A resulting reduction in aerobic activity could negatively affect movement, feeding, reproduction, and growth. The effects of temperature on aerobic scope were studied in Fraser River sockeye salmon (*Oncorhynchus nerka*; Farrell 2009). These fish closely integrate the optimal temperature for maximal aerobic scope with the water temperature during upstream spawning migrations. Higher temperatures could reduce aerobic scope, with negative effects on upstream migration and fecundity, and was in fact observed in 2004, when higher river temperatures decreased aerobic scope, causing a high mortality rate (~70%) in returning adults. Temperature effects on aerobic scope have also been observed in the common eelpout (*Zoarces viviparus*; Portner and Knust 2007).

Growth: Physiological Responses and Growth Indices

Most organisms initially respond to environmental changes through phenotypic plasticity, both physiological and behavioral (Bradshaw and Holzapfel 2010). Organisms rely on phenotypic plasticity as the initial response to environmental variability through adjustment of relevant gene expression (Hofmann and Todgham 2010), as in thermal

acclimation (Farrell 2009). An example could be the maintenance of cell membrane structure and fluidity with changing temperature (Hazel 1995). Intertidal mussels have shown rapid restructuring of cell membrane structure with fluctuations in temperature (Dahlhoff and Somero 1993, Williams and Somero 1996). But phenotypic plasticity can sometimes have energetic costs, with one potential reason a reallocation of resources (Angilletta et al. 2003). While this could result in declines in other functions such as reproduction and growth (Hofmann and Todgham 2010), such trade-offs cannot be considered a universal result of phenotypic plasticity (Portner et al. 2006).

With the wide-ranging effects of temperature and diet, it is important to understand the effects of changes in these factors on growth and condition of Pacific cod, walleye pollock, and Pacific herring. However, there is a relative lack of data on the immediate growth responses of these fish, and their physiological capability to adapt to temperature and nutritional changes, especially in the larval and juvenile stages. Determining the nature of these physiological energetic responses can help in understanding growth and condition in these fish as it relates to temperature and diet.

Numerous physiological indices are currently used in growth studies, including lipid concentrations (Laurel et al. 2010), protein concentrations (Houlihan et al. 1995), enzyme activities (Pelletier et al. 1994), and nucleic acid ratios (RNA/DNA; Tong et al. 2010). A growth index should be a good measure of growth status and condition, and sensitive to changes in growth. Each of these growth indices, however, has certain limitations. Lipid and protein concentrations are very useful in charting growth and body condition over extended periods of time, as in seasonal fish studies (Adams 1999,

Vollenweider et al. 2011). But given the role of lipids as stored energy reserves (Paul and Paul 1999), they do not have as much utility as an instantaneous growth measure. By the nature and consumption of lipids as energetic reserves, there could be a lag between an apparent change in lipid levels and immediate environmental conditions. Protein concentrations are useful in studying somatic growth, but cannot account for varied resource allocation strategies, such as allocation towards lipid storage. Changing seasonal energy allocation strategies are in fact observed in numerous fish species including Pacific herring (Flath and Diana 1985, Post and Evans 1989, Foy and Paul 1999, Paul and Paul 1999). Activities of some enzymes essential to energy metabolism, such as glycolytic enzymes, are positively correlated to growth rate (Pelletier et al. 1993*a*, 1994, 1995), and can be sensitive growth indices. However, it can be hard to determine thermal compensation of enzyme activities (Guderley 2004). In bluefin tuna (*Thunnus thynnus*), activities of certain enzymes in the retia mirabilia, including pyruvate kinase and citrate synthase, are affected by temperature, but activity of other enzymes, such as lactate dehydrogenase, can remain unchanged (Fudge et al. 1997), while glycolytic enzyme activity in white muscle can remain unchanged even with a thermal gradient (Fudge et al. 2001). Variations in the nature of enzyme activity or unchanging enzyme activity in different tissues could affect its use as a growth index in some fish species.

Nucleic acid ratios (R/D) are a sensitive index of instantaneous growth (Buckley 1979, 1984, Busacker et al. 1990, McLaughlin et al. 1995, Buckley et al. 1999, Weber et al. 2003) and a good indicator of immediate environmental conditions (Caldarone et al.

2003). This index has been correlated with growth in numerous fish species (Robinson and Ware 1988, Smith and Buckley 2003, Caldarone 2005, MacLean et al. 2008, Stierhoff et al. 2009). The principle underlying R/D is that while cellular DNA concentrations remain relatively stable, RNA concentrations reflect the amount of protein synthesis required for tissue growth (Buckley 1984, McLaughlin et al. 1995), and are consequently related to current growth as well as nutritional condition (Weber et al. 2003). Concentrations of RNA can decrease during reduced growth and starvation (Buckley et al. 1999), resulting in decreased R/D indicative of growth restrictions. An advantage of R/D is its utility and sensitivity over multiple fish species and tissue types, and essentially any life-stage, ranging from 0 days post hatch larvae through adult stages.

A primary limitation in using R/D is that RNA translational efficiency (amount of protein translated per unit RNA) increases with temperature (Lewis and Driedzic 2007), changing the R/D-growth relationship. Consequently, R/D cannot be directly compared as a growth index between fish sampled at temperatures differing by more than 2°C (Buckley et al. 1999). Use of R/D requires calibrating ratios and growth rates over a temperature range for a given species, namely R/D-Growth-Temperature (R/D-G-T) calibrated models (Buckley 1984). Building these models require laboratory studies, with fish cultured at relevant temperatures and with measured growth rates, which can then be calibrated with measured R/D. These models can have applications in predicting growth in field-sampled fish, and could allow for growth comparisons between fish collected at different temperatures. More importantly, this growth index can be usefully integrated with other relevant physiological indices, such as whole body lipid and protein

concentrations, providing a comprehensive overview of fish condition at a given time. Specifically, using R/D in conjunction with other indices could help in determining energetic responses and growth patterns in Pacific cod, walleye pollock, and Pacific herring, as it relates to temperature and diet.

Summary

Pacific cod, walleye pollock, and Pacific herring are ecologically and commercially important species in the North Pacific Ocean. With the exception of a few studies, there is a dearth of information on energetic assessments and growth parameters in larval and juvenile stages of these fish. Understanding larval and juvenile growth as it relates to temperature and diet can be important since larval growth patterns could determine future growth. Changes in temperature and diet can have effects on these species ranging from the ecosystem level to the molecular level, potentially affecting growth and condition. Determining the physiological responses of these fish to changes in temperature and diet can help in understanding growth and condition. Energetic assessments could also be useful in predictive growth models, potentially aiding stock assessments that require growth and condition estimates. A range of growth indices are useful in determining physiological growth responses. Nucleic acid ratios have potential as a tool in determining growth responses in these fish, and in conjunction with other physiological indices such as lipid and protein concentrations, could provide a comprehensive understanding of growth and condition.

Chapter 1: Nutritional Ecology of Juvenile Pacific Herring (*Clupea pallasii*): Nucleic Acid Ratios as an Index of Growth and Starvation Threshold [1]

Abstract:

Pacific herring (*Clupea pallasii*) is an ecologically important prey and predator species in the North Pacific Ocean that sustains economically and culturally important fisheries, particularly in Prince William Sound, Alaska, where there has been concern after the failure of stocks to regain abundance after a crash in 1993. Annual recruitment can vary significantly between populations and years, making population predictions difficult. Young-of-the-year Pacific herring face critical energetic challenges at the onset of their first winter due to competing needs for energy to support both growth and energy storage. This energetic challenge is intensified by the short summer growing season in the North Pacific Ocean, and is an important determinant of year class success and population abundance. Energetic assessments for young-of-the-year Pacific herring would improve stock assessment models for Pacific herring. We used the nucleic acid ratio (RNA/DNA) from white muscle tissue of young-of-the-year Pacific herring as a measure of growth in fed, starved/fed, and terminally starved fish cultured at 6.5°C, 8.5°C, and 12.5°C. Fish growth in body mass corresponded to changes in RNA/DNA. Relative to fed controls, starved/fed fish showed restored RNA/DNA ratios and soluble protein concentration, but lesser mass. Nucleic acid ratios in starved/fed fish during the starvation phase, and in terminally starved fish, indicated incipient terminal starvation. Seasonal variation of

[1]Ashwin Sreenivasan, Ronald A. Heintz, Johanna J. Vollenweider, Stanley D. Rice, Paul K. Hershberger, Jacob L. Gregg. Nutritional Ecology of Juvenile Pacific Herring (*Clupea pallasii*): Nucleic Acid Ratios as an Index of Growth and Starvation Threshold. Prepared for submission in Marine Ecology Progress Series

RNA/DNA, protein concentrations and total body lipid concentrations in fed fish indicated seasonal changes in resource allocation, growth rates, and RNA/DNA.

Introduction

Pacific herring (*Clupea pallasii*) are arguably the most important forage species in the North Pacific Ocean (Paul et al. 1998), with large biomass ranging from California to Alaska and northeastern Pacific waters off Asia (Haegele and Schweigert 1985, Mecklenburg et al. 2002). Local stock biomass can vary considerably, either through commercial fishing, predation by fish, birds, and marine mammals (Womble et al. 2005, Suryan et al. 2006), or disease (Marty et al. 2010). Recruitment of young-of-the-year (YOY) Pacific herring into the population is also variable and depends largely on surviving the first winter (Paul and Paul 1998), when there are few opportunities to feed. Juvenile Pacific herring must grow rapidly during their first summer to survive predation (Paul et al. 1998). They also must build up energy reserves at the end of summer and early fall, storing enough calories to get through the starvation challenging winter (Paul and Paul 1999). Examining these energetic challenges is crucial to better understanding how environmental factors affect growth and recruitment.

Assessing recruitment is a difficult task. In Pacific herring, the first possible estimate for each cohort has been when juveniles mature and enter the spawning biomass, and are captured in biomass surveys, either acoustically or in age/length assessments made to support population modeling. This occurs at age 3-4+ in northern populations; consequently there is a 3-4 year delay in assessing recruitment success of a year class. In an attempt to get earlier assessment and modeling tools for the Prince William Sound

Pacific herring population, a series of studies have been initiated by US NOAA Fisheries, of which this study is a part. This once economically important population (Paul et al. 1998) has been depressed since a population crash in 1993, which involved year classes affected by the Exxon Valdez oil spill. The reasons for lack of recovery remain unknown (Ashe et al. 2005). Here we report the energetic responses of fed and starved YOY Pacific herring at different temperatures in laboratory experiments, with the objective of providing energetic assessments that can be used in population prediction models.

Energetic responses to Pacific herring's nutritional environment can include compensatory growth (CG), which occurs due to heightened protein synthesis and accretion (Treberg et al. 2005, Smith and Ottema 2006). After a period of depressed growth, most fish undergo CG with the onset of favorable conditions until normal growth trajectories are restored (Ali et al. 2003, Treberg et al. 2005). Compensatory growth may be particularly important to the fitness of high-latitude species like Pacific herring, which experience wide seasonal cycles of light, temperature and prey availability. Given the high metabolic costs of protein synthesis (Kiørboe and Mohlenberg 1987, Jorgensen 1988), a threshold loss in body mass might be required to trigger CG in Pacific herring, as observed in hybrid striped bass (*Morone chrysops* x *Morone saxatilis*; Picha et al. 2006).

Even during prolonged starvation, minimal metabolic functions are required for survival. A growth index that can be used in energetic assessments and also identify the status of an individual fish relative to this "baseline" metabolic level would be useful in nutritional and energetic studies of natural herring populations.

An accurate index of growth responses is nucleic acid ratios (R/D; Buckley 1979, 1984, Busacker et al. 1990, McLaughlin et al. 1995, Buckley et al. 1999, Weber et al. 2003). While cellular DNA concentrations remain stable, RNA concentrations increase or decrease, reflecting protein synthesis (Buckley 1984, McLaughlin et al. 1995), and thus indicate nutritional condition (Weber et al. 2003). While proximate analyses and lipid concentrations are good seasonal indicators of fish condition and health (Adams 1999), R/D ratios are more sensitive and thus responsive to immediate environmental variations (Caldarone et al. 2003).

However, RNA translational efficiency increases with temperature (Lewis and Driedzic 2007), affecting the ratio-growth relationship. This precludes direct R/D comparisons between fish sampled at temperatures >2°C apart (Buckley et al. 1999), requiring a calibration of ratios and growth rates over a temperature range, namely R/D-Growth-Temperature calibrated models (R/D-G-T; Buckley 1984). These models allow direct R/D and growth comparisons between fish sampled at different temperatures (Caldarone et al. 2003).

Our goal was to measure energetic responses at the molecular level in YOY Pacific herring after a period of starvation. In two separate experiments, we compared R/D and soluble protein between fed and starved/fed YOY Pacific herring, and R/D, soluble protein, and total body lipid between fed and terminally starved Pacific herring at each of three temperatures. The aim of measuring R/D was to determine energetic parameters in YOY Pacific herring, and also utilize those parameters in building a predictive growth model. Soluble protein concentrations were examined as protein

synthesis and concentration should respond to variations in R/D ratios; also CG is driven by heightened protein synthesis. Corresponding changes in soluble protein can strengthen the validation of R/D ratios as a growth index in Pacific herring. Lipid is an energy storage fuel, and examining total body lipid in conjunction with R/D and protein can clarify energy allocation strategies, i.e. growth vs energy storage, the balancing of which is crucial to overwinter survival of YOY Pacific herring.

The objectives of this study were to a) determine growth responses in fed and starved/fed YOY Pacific herring using R/D ratios, b) determine growth responses in fed and starved YOY Pacific herring using R/D ratios, and c) develop a predictive growth model for YOY Pacific herring using R/D ratios measured over a range of temperatures and nutritional states.

Materials and Methods

This study included a CG experiment (March-July 2008) and a terminal starvation experiment (August-October 2008). In the CG experiment, Pacific herring were starved for a period and returned to feeding; in the terminal starvation experiment, Pacific herring were starved until death. Young-of-the-year Pacific herring were obtained from Puget Sound, Washington, in November 2007 and July 2008 by the U.S Geological Survey (USGS) Marrowstone Marine Laboratory (MML), Nordland, Washington and cultured there during the experiments.

Compensatory Growth Experiment

To determine if CG was affected by temperature, we randomly assigned fish to three temperature treatments: ambient (8.5°C), warm (12.5°C), and cool (6.5°C), and held them at natural photoperiod from March to July 2008. Water temperatures were measured every 30 minutes by temperature loggers. Each temperature treatment included a control group (fed continuously; fed) and a starved group (fed before and after a period of starvation; starved/fed). Across temperatures, fish in each feeding treatment were held in three tanks (~300 liters capacity) with a flow rate of ~4-8 liters $minute^{-1}$; thus, in total, 18 tanks (9 tanks fed, 9 tanks starved/fed) were used. In the starved/fed group, each tank held 30 fish. In the fed group, each tank held 15 fish. Sampling interval was based on temperature units (one temperature unit accumulates per degree Celsius per day). During the starvation period, fish in all temperature groups were euthanized and sampled (2/tank) every 137.5 temperature units, until ~40% mortality was reached. At this stage, feeding was resumed in the starved/fed groups. All fish in both fed and starved/fed groups were fed daily *ad-libitum* with Bio-Olympic ™ fry feed from Bio-Oregon (2mm pellet). After feeding resumed, fish in both groups across all temperatures were combined into 2 tanks instead of 3. Fish were fed until the termination of the experiment after 61 days (warm group), 88 days (ambient group), and 113 days (cool group), when all remaining fish were sampled.

Fish were measured (weight and length) and white muscle plugs were removed from fish during each sampling period. Sampled fish were euthanized in a solution of tricainemethanesulfonate (MS-222; 0.025g $liter^{-1}$), after which, wet weights (0.001g) and

fork lengths (0.5mm) of fish were measured. White muscle plugs (~50mg) for R/D and soluble protein assays were removed from the musculature below the dorsal fin with a scalpel, placed in individual 1500 μL microcentrifuge vials on ice, and then stored at -80°C until analysis. After muscle plugs were removed, the individual fish were then stored at -20°C until proximate analysis.

Instantaneous growth rates (length-GR_L, weight-GR_W) were calculated for fish in all treatments from one sampling date (t_2) to the preceding date (t_1) using the formula:

$$GR_L = (\ln l_{t2} - \ln l_{t1}) / (t_2 - t_1)$$

$$GR_W = (\ln w_{t2} - \ln w_{t1}) / (t_2 - t_1)$$

where l = mean length (mm), w = mean weight (g), and ln = natural logarithm.

Terminal Starvation Experiment

Fish were cultured in 2 common tanks (~3700 liter capacity each) before being randomly assigned to treatment tanks. During August-October 2008, fish were sampled from the common tanks (18 fish in total) at the beginning of the experiment as a starting point for measuring growth. Fed and starved fish across temperatures were sampled concurrently (~9 fish/group) over most of the study duration. At the termination of the experiment after 59 days (warm treatment), 65 days (ambient treatment), and 68 days (cool treatment), all remaining fish were sampled. All experimental conditions, treatments, and groups were identical to the CG experiment, except that in this experiment fish were starved until the end of the study, i.e. no resumption of feeding. The sampling protocol was identical to the compensatory growth experiment.

Biochemical Analyses

Compensatory Growth Experiment

All R/D ratios were measured by a one dye-two enzyme (RNase and DNase) fluorometric protocol modified from Caldarone et al. (2001). Muscle plugs were subdivided, and ~30mg tissue samples were placed in individual 1500 µL microcentrifuge vials with 300µL 2% N-lauroylsarcosine Tris-EDTA buffer, and sonicated using a Branson Sonifier 250 (VWR Scientific, Radnor, PA). Samples were then vortexed for 60 m, further diluted with 1200µL Tris-EDTA buffer, and centrifuged for 15 m at 14000 g using a Sorvall Legend Micro 21 R refrigerated centrifuge (Thermo Scientific, Waltham, MA). Supernatants were then treated with 75µL ethidium bromide (5µg ml^{-1}) according to the protocol outlined by Caldarone et al. (2001). Total fluorescence was measured using a Wallac 1420 microplate spectrophotometer (Perkin Elmer, Waltham, MA) at excitation and emission wavelengths of 355nm and 600nm respectively. Samples were sequentially treated with RNase and DNase, and the resulting reduced fluorescence measured to obtain RNA and DNA fluorescence respectively. Calibration curves were constructed using serial dilutions of 18s-28s rRNA (Sigma R-0889) and calf thymus DNA (Sigma D-4764) standards. Supernatants for R/D were read on Corning NBS 96-well black flat-bottom microplates (75µL samples). Every R/D sample was run in three individual wells. Fluorescence values for all three wells were read four times, and the coefficient of variation associated with four reads was examined. When the variation was higher than 10%, the individual well fluorescence values were

examined, and the data point causing the high coefficient of variation was excluded from the analysis.

The remaining supernatant was saved for protein analysis and stored at -20°C. Soluble protein concentrations (μg/mg tissue) were measured using a 660nm protein assay (Pierce Scientific, Rockford, IL). All protein samples were read on 96-well clear round-bottom microplates (10μL samples). Each protein sample was run in duplicate.

Terminal Starvation Experiment

All samples were analyzed for R/D ratios and protein using identical protocols as in the CG experiment. All sampled fish were retained for proximate analysis, and total body lipid was analyzed to examine resource partitioning. Lipid analysis was carried out according to a protocol modified from the Folch's method, outlined by Christie (1982). Approximately ~0.5g wet sample homogenate was placed in a Dionex Accelerated Solvent Extractor 200, and lipid extracted using 2:1 (v:v) chloroform:methanol. All extracts were then sequentially washed with 0.88% KCl and 1:1 (v:v) methanol:deionized water in a volume that equaled 25% of extract volume. The excess solvent was then evaporated and percent lipid values were gravimetrically determined. Because it was not feasible to analyze total body lipid in all samples, samples from all treatments and sampling dates were randomly chosen, a total of 65 fish. Samples were taken such that the entire experimental duration was covered for each treatment (i.e., lipid content was determined from fish at the start, approximate middle, and end of the experiments).

Statistical Analyses

In all analyses, differences between groups were considered significant when $p \leq 0.05$

Compensatory Growth Experiment

Growth was compared between fed and starved/fed groups. A General Linear Model (GLM) including a Tukey's posthoc test was used to compare final weights and lengths of fish (measured at the end of the experiment) between fed and starved/fed groups in all treatments, with weight or length as the dependent variable, and temperature treatment and feeding treatment as independent variables. A GLM with a Tukey's posthoc test was used to compare final soluble protein concentrations and R/D ratios of fed groups in all treatments (measured at the end of the experiment) to final values in starved/fed fish, with protein or R/D ratios as the dependent variable, and temperature treatment and feeding treatment as independent variables. Soluble protein concentrations and R/D ratios of starved/fed fish at resumption of feeding were compared to final R/D and protein values using two sample T-tests. A GLM was used to compare R/D ratios of starved/fed fish up to the resumption of feeding in all temperature treatments, with R/D as the dependent value, and temperature treatment and sampling day as independent variables. All statistical analyses were made with Minitab v. 14 (Minitab Inc., State College, Pennsylvania).

Terminal Starvation Experiment

Growth was compared between fed and starved fish. Weights and lengths of fed and starved fish within temperature treatments were compared by two sample T-tests. Growth rates (weight and length) were compared between fed and starved fish within treatments by two sample T-tests. A GLM was used to compare R/D ratios of fed and starved fish within individual temperature treatments, with R/D as the dependent value and sampling day and fed/starved treatments as independent variables. A GLM was used to compare soluble protein concentrations of fed and starved fish within treatments, with protein as the dependent variable and sampling day and fed/starved treatments as independent variables. A GLM was used to compare total body lipid percent values of starved fish within treatments, with percent lipid as the dependent variable and sampling day as the independent variable. A GLM was used to compare R/D ratios of starved fish between treatments, with R/D as the dependent value, and temperature treatment and sampling day as independent variables. Nucleic acid ratios of starved fish in individual treatments between both experiments were compared by two sample T-Tests. All statistical analyses were made with Minitab v. 14 (Minitab Inc., State College, Pennsylvania).

Growth Model

Multiple R/D-G-T calibrated models were generated to best describe the relationship between instantaneous growth rates (IGR), and variables including R/D, temperature, mass, and an R/D*Temperature interaction factor. The R/D-G-T models for juvenile herring were generated through multiple linear regressions. Akaike's

information criterion (AIC) was used to select the best fit model from the model data set (Wagenmakers and Farrell 2004). The regression models were generated by Minitab v. 14 (Minitab Inc., State College, Pennsylvania).

Results

Compensatory Growth Experiment

A total of 104 fish were analyzed in this experiment. Body mass (g) and length (mm) of all starved fish increased after the resumption of feeding, but did not reach that of fed controls. Fed controls over all temperature treatments had higher body mass (cool treatment 10.57g ± 0.9935; ambient treatment 10.70g ± 1.19; warm treatment 11.68g ± 1.13) than starved/fed fish (cool treatment 7.57g ± 0.84; ambient treatment 6.65g ± 0.44; warm treatment 7.80g ± 0.68) at the end of the experiment ($p < 0.001$; Fig 1.1a; Table 1.1); however there were no statistically significant differences between fed and starved/fed groups at any one temperature (cool treatment $p = 0.4711$; ambient treatment $p = 0.0867$; warm treatment $p = 0.0992$; Table 1.1). A similar pattern was seen in fish length; fed controls over all treatments were also longer (cool treatment 102.5mm ± 2.50; ambient treatment 103.75mm ± 2.99; warm treatment 102.42mm ± 2.87) than starved/fed groups (cool treatment 97mm ± 2.09; ambient treatment 95.43mm ± 1.68; warm treatment 94.05mm ± 1.96) at the end of the experiment ($p = 0.004$; Table 1.1); there were no statistically significant differences between groups within any temperature treatment (cool treatment $p = 0.9316$; ambient treatment $p = 0.1939$; warm treatment $p = 0.3726$; Table 1.1).

Soluble protein concentration and R/D increased across all temperature treatments after resumption of feeding (Figs. 1.1b, 1.1c). At the end of the experiment, no significant difference was detected between fed and starved/fed fish, suggesting a healthy recovery from the starvation period. Soluble protein concentrations ($\mu g\ mg^{-1}$) rapidly rose in starved/fed fish after resumption of feeding, approximately doubling by the end of the experiment (Fig. 1.1b). There was no difference in protein concentrations at the end of the experiment between fed (cool treatment 39.13 $\mu g\ mg^{-1} \pm 3.65$; ambient treatment 35.15 $\mu g\ mg^{-1} \pm 4.18$; warm treatment 104.85 $\mu g\ mg^{-1} \pm 4.52$) and starved/fed groups (cool treatment 44.04 $\mu g\ mg^{-1} \pm 5.05$; ambient treatment 51.93 $\mu g\ mg^{-1} \pm 7.82$; warm treatment 56.29 $\mu g\ mg^{-1} \pm 6.51$) over all treatments ($p = 0.227$; Table 1.1); however a significant difference was detected between fed and starved/fed groups in the warm treatment (cool treatment $p = 0.9037$; ambient treatment $p = 0.4690$; warm treatment $p < 0.001$; Table 1.1). Final protein values were higher than values at resumption of feeding in starved fish (cool treatment $p = 0.032$, ambient treatment $p = 0.017$, hot treatment $p = 0.000$).

Starved/fed fish had rapid increases in R/D ratios, approximately doubling their ratios after resumption of feeding, by the end of the experiment (Fig. 1.1c). There was no difference in R/D at the end of the experiment between fed (cool treatment 7.53 ± 0.37; ambient treatment 6.89 ± 0.55; warm treatment 7.58 ± 0.46) and starved/fed groups (cool treatment 8.43 ± 0.64; ambient treatment 7.83 ± 0.46; warm treatment 6.94 ± 0.62) over all treatments ($p = 0.380$; Table 1.1); there were no significant differences between groups within any treatment (cool treatment $p = 0.8836$; ambient treatment $p = 0.8546$;

warm treatment $p = 0.9320$; Table 1.1). Final R/D ratios were higher than R/D at resumption of feeding in starved fish (cold treatment $p = 0.001$, ambient and hot treatment $p = 0.000$). Nucleic acid ratios of starved/fed fish until resumption of feeding (baseline values) were not different between temperature treatments ($p = 0.277$).

Terminal Starvation Experiment

A total of 330 fish were analyzed in this experiment. Starved fish in the cool treatment lost approximately 25% of their body mass over the experimental sampling period of 44 days (Fig. 1.2a); survival was affected. Similar results were observed in the ambient and warm treatments (Figs. 1.3a, 1.4a), with a faster rate of mass loss in the warm treatment. Body mass and length were significantly greater in fed fish; fed fish in all treatments had higher mass ($p < 0.001$) than starved fish (Figs. 1.2a, 1.3a, 1.4a) and were longer in cool ($p = 0.004$), ambient ($p < 0.001$) and warm treatments ($p < 0.001$). Fed fish had significantly higher GR_W in all treatments (cold and ambient treatment $p = 0.001$, hot treatment $p = 0.018$). No differences in GR_L were found between fed and starved fish in cool ($p = 0.258$) and ambient treatments ($p = 0.263$), while GR_L of fed fish in warm treatment was higher ($p = 0.025$).

The declines in RNA/DNA and protein in starved fish were evident by the first sampling, indicating the rapid response in these indices. As expected, the nucleic acid ratios, lipid, and protein values in all fed fish were higher than starved fish. Nucleic acid ratios in fed fish were higher compared to starved fish in all three temperature treatments ($p < 0.001$) (Figs. 1.2b, 1.3b, 1.4b). Fed fish in all treatments had temporally depressed and elevated R/D. In contrast, R/D in all starved fish remained low after the 1st sampling.

The trend observed in soluble protein ($\mu g\ mg^{-1}$) did not correspond with that of R/D ratios. Across all treatments, protein levels in starved and fed fish remained at nearly identical levels, dropping steadily until approximately midpoint through the sampling. At this stage, corresponding to the initial spike in R/D, protein levels in fed fish increased (Figs. 1.2b, 1.3b, 1.4b). Protein values were higher in the fed fish in cool, ambient, and warm treatments ($p < 0.001$). Significant reductions in total body lipid percent values were found for starved fish in the ambient ($p = 0.004$) and warm ($p < 0.001$) treatments, but not in the cool treatment ($p = 0.514$) (Figs. 1.5a, b, c).

Baseline R/D values of starved fish across treatments were similar. No difference was detected across ambient, cool, and warm treatments ($p = 0.506$). A comparison of baseline R/D ratios for starved fish across the CG and terminal starvation experiments revealed a difference between values for starved fish in the warm treatment (CG experiment mean R/D = 3.4601, terminal starvation experiment mean R/D = 3.0947; $p = 0.034$) but no differences between values in the ambient (CG experiment mean R/D = 4.3509, terminal starvation experiment mean R/D = 4.3241; $p = 0.918$) and cool treatments (CG experiment mean R/D = 4.2038, terminal starvation experiment mean R/D = 4.0513; $p= 0.520$).

Growth model synthesis

A significant positive relationship ($p < 0.001$) was found between instantaneous growth rates and mean R/D ratios, temperature, and mean mass (Table 1.2). The growth model selected from the model data set based on a comparison of model AIC values (Table 1.3) incorporated mean R/D, mean mass, and treatment temperature from fish in

both experiments as predictors of instantaneous growth rate (Table 1.2). All parameters included in the model were significant except for temperature (Table 1.2). Growth rates predicted by the model closely matched observed growth rates in all treatments (Figs. 1.6a, b, c).

Discussion

Compensatory Growth Experiment

Starved/fed fish demonstrated marked compensation in R/D and protein, but had lower body mass than fed controls. This could be due to termination of the experiment before mass in starved/fed fish could increase to that of fed controls. Additionally, there could be a lag between heightened R/D ratios and protein synthesis, and actual protein accretion resulting in increased muscle mass. Even though compensation in mass was limited, heightened protein synthesis in white muscle is seen during initial stages of growth recovery in other fish species (Lewis and Driedzic 2007). Since CG is characterized by heightened protein synthesis (Smith and Ottema 2006), the compensation in R/D and protein in white muscle of starved/fed herring suggests the onset of CG. The rising soluble protein levels in starved/fed fish also probably reflect increased synthesis of growth-related metabolic enzymes. Such an increase in enzyme synthesis would directly follow higher R/D levels, without immediately causing an increase in body mass.

Compensation in R/D and protein was not impaired within any temperature treatment. Starved/fed fish within individual temperature treatments had final R/D and protein values similar to that of fed controls. This suggests that YOY Pacific herring

might recover from fasting over a range of temperatures. Protein synthesis rates in fish can be flexible after temperature acclimation (Treberg et al. 2005). Flexibility in protein synthesis could allow continued growth in these aquatic ectotherms, even with small fluctuations in temperature.

Compensatory growth should be examined in terms of metabolic and developmental costs. Since normal fish growth is well below accelerated CG rates (Ali et al. 2003), it must be that the fitness cost of prolonged CG rates is unacceptably high under normal conditions (Arendt 1997, Metcalfe and Monaghan 2001). These physiological costs of fitness are mainly due to the synthesis of protein (Jorgensen 1988) and precursors, including nucleotides for RNA (Bocharova et al. 1992). In spite of cost-mitigation strategies (Houlihan et al. 1988, Houlihan et al. 1995, Wieser 1995, Smith et al. 2000, Smith and Ottema 2006), protein and RNA synthesis contribute substantially to metabolic rate (Houlihan et al. 1995, Smith and Houlihan 1995, Smith and Ottema 2006) with some estimates ranging from ~18-26% for protein and ~10% for RNA synthesis (Hawkins 1991, Guppy et al. 1994).

Extended CG can also disrupt normal energy allocation patterns, with developmental effects. Extended CG depresses fecundity (Ali et al. 2003), digestion (Pedersen et al. 1990), muscle development and locomotion (Christiansen et al. 1992, Billerbeck et al. 2001, Sogard and Olla 2002, Royle et al. 2006), and immunocompetence (Arendt 1997). Other abnormalities associated with extended CG range from cranial structure development (Arendt and Wilson 2000) to scale integrity and strength (Arendt et al. 2001). Negative effects of CG may not be immediately apparent, but may cause

delayed mortality in fish (Johnsson and Bohlin 2006). With these associated metabolic and developmental costs, a shift in the extent of CG could affect fitness. A prolonged period of fasting, for instance due to delayed food availability, could intensify the extent of CG required to normalize growth trajectories, but with higher metabolic and developmental costs.

Compensatory growth in fish also does not occur after extremely long periods of starvation, i.e., there is a threshold below which no recovery is possible and mortality occurs (Ryan 1990, Ali et al. 2003). This growth/mortality threshold may also be present as a "baseline" R/D ratio in starved Pacific herring, one required for maintenance metabolism and survival. The stable R/D (~4-5) in the starved/fed groups observed here during the starvation period may indicate a baseline R/D for YOY Pacific herring. Trends in protein synthesis rates similar to those observed here have been found in starving Atlantic herring (*Clupea harengus*; Houlihan et al. 1995) and Atlantic cod (*Gadus morhua*; Houlihan et al. 1988), suggesting a minimum amount of protein synthesis required for survival. A baseline R/D ratio corresponding to this minimal protein synthesis could represent a growth/mortality threshold, such that fish with R/D values lower than baseline would not recover from starvation. While requiring further validation, these initial results suggest the potential uses of a baseline R/D ratio as a rapid condition index in YOY herring.

Pacific herring in this experiment underwent marked compensation in R/D and protein, with limited compensation in body mass by the end of the experiment. Nucleic acid ratios showed compensation in steps that precede and could be essential to CG,

which is an increase in mass. These initial results show that R/D ratios can be useful as a tool in observing growth responses in YOY herring. With further development, R/D ratios could provide relatively rapid measures of condition in these fish. These results have to be interpreted with the caveat that they cannot be generally extended to older age classes or other populations of Pacific herring without further research.

Terminal Starvation Experiment

In all treatments, R/D in fed fish was higher, but decreased from approximately mid-August through mid-September, implying a seasonal effect. The seasonal effect could be the influence of changing photoperiod on resource allocation and partitioning (Foy and Paul 1999), i.e., between growth and lipid storage (Paul and Paul 1999). Similar seasonal energetic strategies are seen in other cold water fish (Flath and Diana 1985, Post and Evans 1989).

Herring normally allocate resources to lipid storage during late summer and early fall, to build energy reserves for winter (Blaxter and Holiday 1963, Paul and Paul 1998). Lipids are the main source of stored energy in Pacific herring, while protein synthesis is primarily responsible for somatic growth (Foy and Paul 1999). With reducing day length, most resources are allocated to building lipid reserves, at the expense of somatic growth (Paul and Paul 1999). The decreasing R/D in fed fish observed here could indicate seasonal reduction in somatic growth and protein synthesis; it is consistent with the simultaneous increase observed in total body lipid. The lowest point in R/D ratios in fed fish (mid-September) corresponded to ~6% total body lipid in all treatments. Nucleic

acid ratios and protein started increasing at this point in fed fish, suggesting resumption of somatic growth after fish reached a lipid threshold.

Soluble protein concentrations in starved groups did not consistently mirror R/D ratios. Towards the end of the experiment, increased protein levels were observed in starved fish in all treatments, but corresponding R/D ratios remained low. This contrasting trend between R/D and protein in starved fish could be due to the effects of terminal starvation. Depending on the temperature treatment, fish had undergone complete starvation for up to 44 days. Nucleic acid ratios remained stable and low in surviving fish, reflecting reduced protein synthesis during starvation. However, starvation in vertebrates proceeds in stages (Stryer 1988, Castellini and Rea 1992). At the terminal stage of starvation, increased protein mobilization occurs after utilization of other endogenous energy sources (Castellini and Rea 1992), including lipids. The spike in soluble protein levels could then correspond to terminal starvation, with muscle protein mobilization after exhaustion of other energy sources. Correspondingly, total body lipid in starved fish dropped over this period (~1.5%), suggesting complete lipid utilization (unpublished herring starvation data; Ronald Heintz). An alternate explanation for rising protein concentrations with stable R/D could be increased RNA translational efficiency (Treberg et al. 2005), with more protein translated per unit RNA. But this is normally observed in rapidly growing fish (Treberg et al. 2005, Lewis and Driedzic 2007) and not in terminally starved fish.

The best growth model predicted ~62% of the observed variance in growth rates of juvenile Pacific herring across treatments (Table 1.3). Nucleic acid ratios, treatment

temperature, and mass were the model parameters that had the highest correlation with instantaneous growth rates. The fact that temperature was not a significant parameter ($p = 0.062$) in the growth model could be due to temperature acclimation in Pacific herring. Protein synthesis in fish can be flexible with temperature acclimation; temperature-independent protein synthesis rates have been found in common carp (*Cyprinus carpio*; Watt et al. 1988), Atlantic cod (*Gadus morhua*; Foster et al. 1992) and toadfish (*Opsanus tau*; Haschemeyer 1968). Protein synthesis rates may thus be conserved independent of temperature (Treberg et al. 2005), through compensatory changes including RNA translational efficiency and capacity. This model may have applications in predicting growth rates of individual Pacific herring. It would only be directly usable with R/D obtained through the fluorometric assay method (Caldarone et al. 2001). In lieu of that, an intercalibration measure (Caldarone et al. 2006) could be used if R/D values were obtained through other experimental methods.

Nucleic acid ratios, soluble protein, and total body lipid of fed herring in this experiment appeared to exhibit seasonal trends. It should be noted that the total body lipid percent values, due to experimental constraints, were obtained from relatively small sample sizes. The stable and low R/D ratios in starved fish could represent a depressed nutritional state similar to that observed in the CG experiment. Further development and validation of a baseline R/D ratio could provide a useful condition index in YOY Pacific herring.

Conclusion

Young-of-the-year Pacific herring showed marked compensation in R/D and protein after a period of starvation. However, they had lower mass than fed controls at the end of the experiment. The lower mass could be due to early termination of the experiment, and possibly a lag between protein synthesis and protein accretion, that would cause an increase in mass. However, the compensation in R/D and protein in white muscle of Pacific herring suggests the onset of CG, since CG is characterized by heightened protein synthesis and accretion in white muscle (Smith and Ottema 2006, Lewis and Driedzic 2007). The benefits of higher growth and increased size in YOY Pacific herring include reduced predation pressure, as well as optimal prey size selection (Paul et al. 1998). The use of R/D ratios was able to highlight compensation in steps that precede and are essential to CG. Nucleic acid ratios have potential as an index in terms of growth/mortality thresholds, and can be useful in determining early growth responses in YOY Pacific herring.

In fed Pacific herring, trends in R/D, soluble protein, and total body lipid suggest a photoperiod-influenced seasonal change of resource allocation and partitioning. With decreasing day length, resources appear allocated to lipid synthesis and storage at the expense of somatic growth. The data indicates resumption of somatic growth after herring reach a potential lipid threshold (~6%).

The Pacific herring growth model based on nucleic acid ratios may have applications in predicting growth rates of individual fish without prior condition

measures. Nucleic acid ratios have applications in determining growth responses in herring, and can complement other growth and condition measures.

Acknowledgements

We thank Elaine Caldarone, who was very generous with her time in helping to adapt her R/D protocol to this research, as well as Dr. Franz Mueter, University of Alaska Fairbanks, and Dr. Chris Hay-Jahans, University of Alaska Southeast, for their advice on statistical methods. We also thank Dr. Sherry Tamone, University of Alaska Southeast, the Rasmuson Fisheries Foundation for fellowship support awarded to AS, the Exxon Valdez Oil Spill Trustee Council for financial support, and NOAA, who supported this research. Experiments involving live vertebrate test animals were performed under an approved Institutional Animal Care and Use Committee protocol from the USGS-Western Fisheries Research Center (Protocol#2008-14).

Literature Cited

Adams MS (1999) Ecological role of lipids in the health and success of fish populations. In: Arts TM, Wainman BC (eds) Lipids in Freshwater Ecosystems. Springer-Verlag, New York: 132-160.

Ali M, Nicieza A, Wootton RJ (2003) Compensatory growth in fishes: a response to growth depression. Fish and Fisheries 4: 147-190.

Arendt JD (1997) Adaptive intrinsic growth rates: an integration across taxa. Q Rev Biol 72: 149-177.

Arendt JD, Wilson DS (2000) Population differences in the onset of cranial ossification in pumpkinseed (*Lepomis gibbosus*), a potential cost of growth. Can J Fish Aquat Sci 57: 351-356.

Arendt JD, Wilson DS, Starck E (2001) Scale strength as a cost of rapid growth in sunfish. Oikos 93: 95-100.

Ashe D, Gray D, Lewis B, Moffitt S, Merizon R (2005) Prince William Sound Management Area 2004 Annual Finfish Management Report. Alaska Department of Fish and Game, Divisions of Sport Fish and Commercial Fisheries: 05-65

Billerbeck JM, Lankford Jr. TE, Conover D (2001) Evolution of intrinsic growth and energy acquisition rates. Part 1. Trade-offs with swimming performance in *Menidia menidia.* Evolution 55: 1863-1872.

Blaxter JHS, Holliday FGT (1963) The behaviour and physiology of herring and other clupeids. Adv Mar Biol 1: 261-393.

Bocharova LS, Gordon RY, Arkhipov VI (1992) Uridine uptake and RNA synthesis in the brain of torpid and awakened ground squirrels. Comp Biochem Physiol B 101: 189-192.

Buckley LJ (1979) Relationships between RNA-DNA ratio, prey density, and growth rate in Atlantic cod (*Gadus morhua*) larvae. J Fish Res Board Can 36: 1497-1502.

Buckley LJ (1984) RNA-DNA ratio: an index of larval fish growth in the sea. Mar Biol 80: 291-298.

Buckley LJ, Caldarone E, Ong TL (1999) RNA-DNA ratio and other nucleic acid-based indicators for growth and condition of marine fishes. Hydrobiologia 401: 265-277.

Busacker GP, Adelman IR, Goolish EM (1990) Growth. In *Methods for Fish Biology* (eds. Schreck CB and Moyle PB), pp 363-388. American Fisheries Society, Bethesda, Maryland.

Caldarone EM, Clemmesen CM, Berdalet E, Miller TJ, Folkvord A, Holt GJ, Olivar MP, Suthers IM (2006) Intercalibration of four spectrofluorometric protocols for measuring RNA/DNA ratios in larval and juvenile fish. Limnol Oceanogr Methods 4: 153-163.

Caldarone EM, St. Onge-Burns JM, Buckley LJ (2001) Protocol and guide for estimating nucleic acids in larval fish using a fluorescence microplate reader. Northeast Fisheries Science Center Reference Document 01-11.

Caldarone, EM, St. Onge-Burns JM, Buckley LJ (2003) Relationship of RNA/DNA ratio and temperature to growth in larvae of Atlantic cod *Gadus morhua*. Mar Ecol Prog Ser 262: 229-240.

Castellini MA, Rea LD (1992) The biochemistry of natural fasting at its limits. Experientia 48: 575-582.

Christie WW (1982) Lipid Analysis: isolation, separation, identification, and structural analysis of lipids. 2nd ed. Pergamon Press, New York.

Christiansen JS, Martinez I, Jobling M, Amin AB (1992) Rapid somatic growth and muscle damage in a salmonid fish. Basic and Applied Myology 2: 235-239.

Flath LE, Diana JS (1985) Seasonal energy dynamics of the alewife in southeastern Lake Michigan. Trans Am Fish Soc 114: 328-337.

Foster AR, Houlihan DF, Hall SJ, Burren LJ (1992) The effect of temperature acclimation on protein synthesis rates and nucleic acid content of juvenile cod (*Gadus morhua* L.). Can J Zool 70: 2095-2102.

Foy RJ, Paul AJ (1999) Winter feeding and changes in somatic energy content of age-0 Pacific herring in Prince William Sound, Alaska. Trans Am Fish Soc 128: 1193-1200.

Guppy M, Fuery CJ, Flanigan JE (1994) Biochemical principles of metabolic depression. Comp Biochem Physiol B109: 175-189.

Haegele CW, Schweigert JF (1985) Distribution and characteristics of herring spawning grounds and description of spawning behavior. Can J Fish Aquat Sci 42 (Suppl 1): 39-55.

Haschemeyer AEV (1968) Compensation of liver protein synthesis in temperature-acclimated toadfish, *Opsanus tau.* Biol Bull 135: 130-140.

Hawkins AJS (1991) Protein turnover: a functional appraisal. Funct Ecol 5: 222-233.

Houlihan DF, Hall SJ, Gray C, Noble BS (1988) Growth rates and protein turnover in Atlantic cod, *Gadus morhua.* Can J Fish Aquat Sci 45: 951-964.

Houlihan DF, Pedersen BH, Steffensen JF, Brechin J (1995) Protein synthesis, growth, and energetics in larval herring (*Clupea harengus*) at different feeding regimes. Fish Physiol Biochem 14: 195-208.

Johnsson JI, Bohlin T (2006) The cost of catching up: increased winter mortality following structural growth compensation in the wild. Proc R Soc B 273: 1281-1286.

Jorgensen CB (1988) Metabolic costs of growth and maintenance in the toad, *Bufo Bufo.* J Exp Biol 138: 319-331.

Kiørboe T, Mohlenberg F (1987) Partitioning of oxygen consumption between "maintenance" and "growth" in developing herring *Clupea harengus* (L.) embryos. J Exp Mar Biol Ecol 111: 99-108.

Lewis JM, Driedzic WR (2007) Tissue specific changes in protein synthesis associated with seasonal metabolic depression and recovery in the north temperate labrid, *Tautogolabrus adspersus.* Am J Physiol-Reg Integr Comp Physiol 293: R474-R481.

Marty GD, Hulson P-J F, Miller SE, Quinn II TJ, Moffitt SD, Merizon RA (2010) Failure of population recovery in relation to disease in Pacific herring. Dis Aquat Org 90: 1-14.

McLaughlin RL, Ferguson MM, Noakes DLG (1995) Concentrations of nucleic acids and protein as indices of nutritional status for recently emerged brook trout (*Salvelinus fontinalis*). Can J Fish Aquat Sci 52: 848-854.

Mecklenburg CW, Mecklenburg TA, Thorsteinson LK (2002) Fishes of Alaska. American Fisheries Society, Maryland.

Metcalfe NB, Monaghan P (2001) Compensation for a bad start: grow now, pay later? Trends Ecol Evol 16: 254-260.

Paul AJ, Paul JM (1998) Comparisons of whole body energy content of captive fasting age-0 Alaskan Pacific Herring (*Clupea pallasi* Valenciennes) and cohorts over-wintering in nature. J Exp Mar Biol Ecol 226: 75-86.

Paul AJ, Paul JM (1999) Interannual and regional variations in body length, weight, and energy content of age-0 Pacific herring from Prince William Sound, Alaska. J Fish Biol 54: 996-1001.

Paul AJ, Paul JM, Brown ED (1998) Fall and spring somatic energy content for Alaskan Pacific herring (*Clupea pallasi* Valenciennes 1847) relative to age, size and sex. J Exp Mar Biol Ecol 223: 133-142.

Pederson BH, Ugelstad I, Hjelmeland K (1990) Effects of a transitory, low food supply in the early life of larval herring (*Clupea harengus*) on mortality, growth, and digestive capacity. Mar Biol 107: 61-66.

Picha EM, Turano MJ, Beckman BR, Borski RJ (2008) Endocrine Biomarkers of growth and applications to aquaculture: A minireview of growth hormone, insulin-like growth factor (IGF)-I and IGF-binding proteins as potential growth indicators in fish. North Am J Aquac 70: 196-211.

Post JR, Evans DO (1989) Size-dependent overwinter mortality of young-of-the-year yellow perch (*Perca flavescens*): laboratory, in situ enclosure, and field experiments. Can J Fish Aquat Sci 46: 1958-1968.

Royle NJ, Lindstrom J, Metcalfe NB (2006) Effect of growth compensation on subsequent physical fitness in green swordtails *Xiphophorus helleri*. Biol Lett 2: 39-42.

Ryan WJ (1990) Compensatory growth in cattle and sheep. Nutr Abstr Rev B 60: 653-664.

Smith RW, Houlihan DF (1995) Protein synthesis and oxygen consumption in fish cells. J Comp Physiol B 165: 93-101.

Smith RW, Ottema C (2006) Growth, oxygen consumption, and protein and RNA synthesis rates in the yolk sac larvae of the African catfish (*Clarias gariepinus*). Comp Biochem Physiol A 143: 315-325.

Smith RW, Palmer RM, Houlihan DF (2000) RNA turnover and protein synthesis in fish cells. J Comp Physiol B 170: 135-144.

Sogard SM, Olla BL (2002) Contrasts in the capacity and underlying mechanisms for compensatory growth in two pelagic marine fishes. Mar Ecol Prog ser 243: 165-177.

Stryer L (1988) Biochemistry (3rd edition). W.H. Freeman and Company/New York.

Suryan RM, Irons DB, Brown ED, Jodice PGR, Roby DD (2006) Site-specific effects on productivity of an upper trophic-level marine predator: Bottom-up, top-down, and mismatch effects on reproduction in a colonial seabird. Prog Oceanogr 68: 303-328.

Treberg JR, Hall JR, Driedzic WR (2005) Enhanced protein synthetic capacity in Atlantic cod (*Gadus morhua*) is associated with temperature-induced compensatory growth. Am J Physiol-Reg Integr Comp Physiol 288: R205-R211.

Vollenweider JJ, Heintz RA, Schaufler L, Bradshaw R (2011) Seasonal cycles in whole-body proximate composition and energy content of forage fish vary with water depth. Mar Biol 158: 413-427.

Wagenmakers EJ, Farrell S (2004) AIC model selection using Akaike weights. Psychon Bull Rev 11: 192-196.

Watt PW, Marshall PA, Heap SP, Loughna PT, Goldspink G (1988) Protein synthesis in tissues of fed and starved carp, acclimated to different temperatures. Fish Physiol Biochem 4: 165-173.

Weber LP, Higgins PS, Carlson RI, Janz DM (2003) Development and validation of methods for measuring multiple biochemical indices of condition in juvenile fishes. J Fish Biol 63: 637-658.

Wieser W (1995) Energetics of fish larvae, the smallest vertebrates. Acta Physiol Scand 154: 279-290.

Womble JN, Willson MF, Sigler MF, Kelly BP, VanBlaricom GR (2005) Distribution of Steller sea lions *Eumetopias jubatus* in relation to spring-spawning fish in SE Alaska. Mar Ecol Prog Ser 294: 271-282.

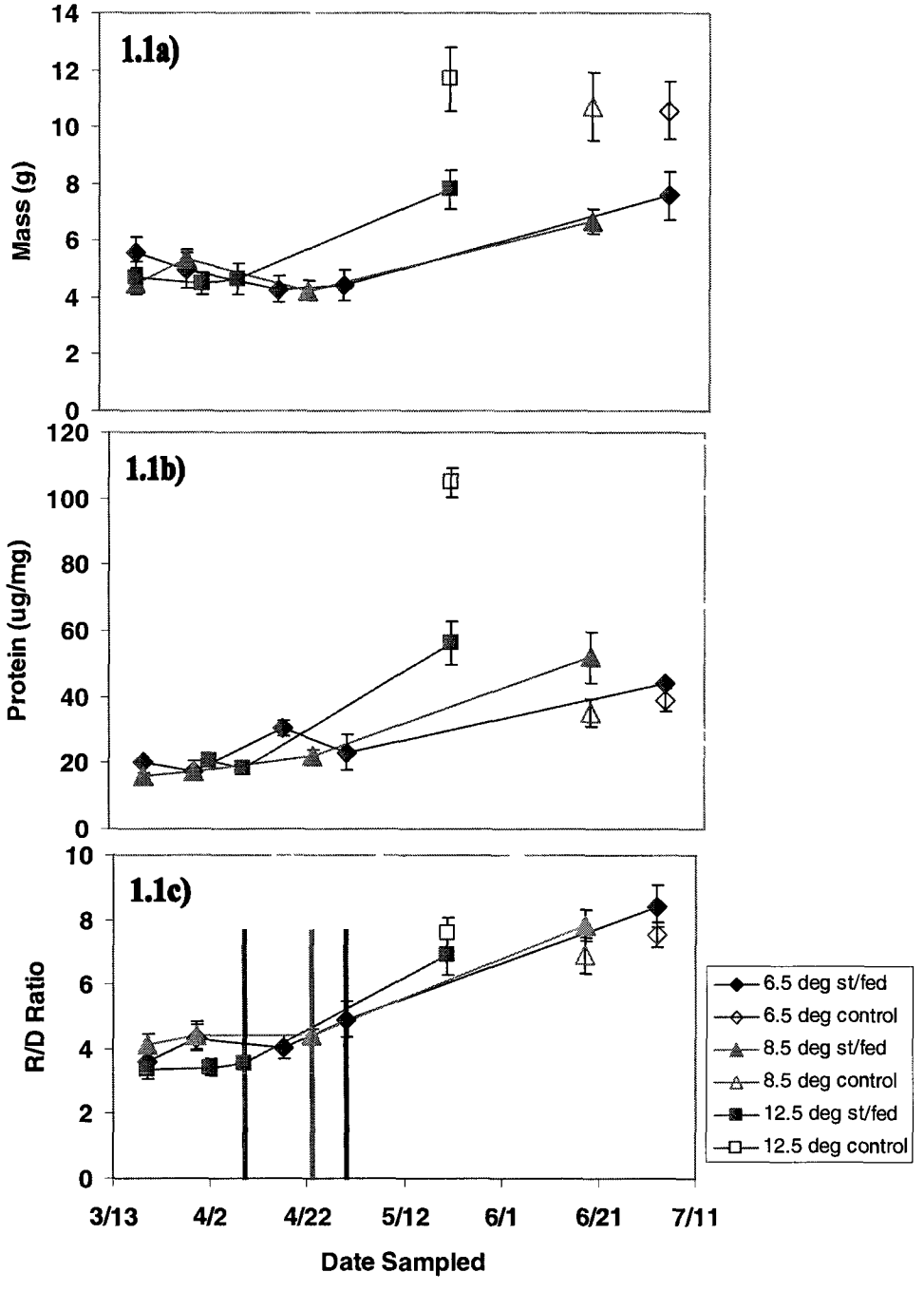

Fig. 1.1a,b,c: *Clupea pallasii*. Mass, protein, RNA/DNA in fed and starved/fed fish. Comparison between body mass (1.1a), protein (1.1b), and RNA/DNA (R/D; 1.1c) of fed controls (open symbols) and starved (st)/fed young-of-the-year Pacific herring (closed symbols) over 6.5°C, 8.5°C, and 12.5°C. Vertical lines represent resumption of feeding in starved fish. Symbol bars represent standard error.

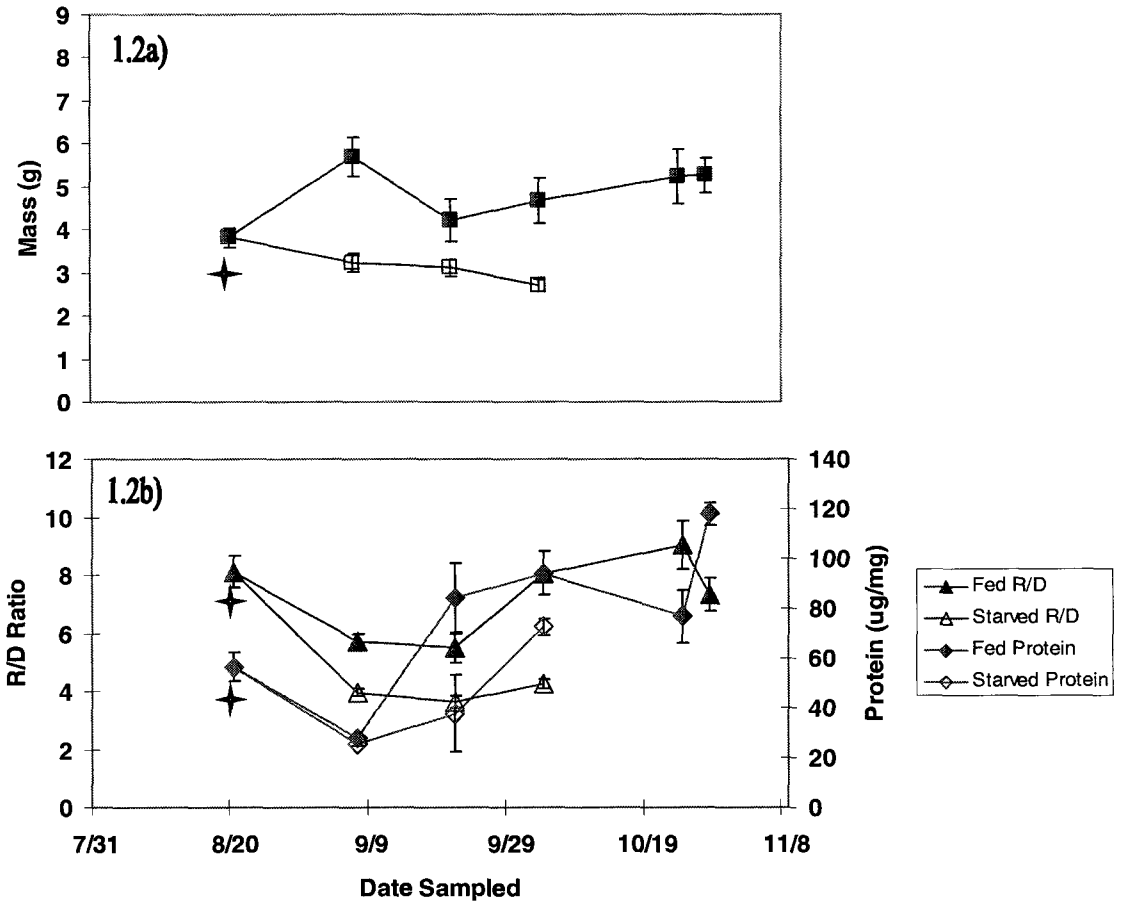

Fig. 1.2a,b: *Clupea pallasii*. Mass, protein, RNA/DNA in cold treatment. Comparison between mass (1.2a), protein (1.2b), and RNA/DNA (R/D; 1.2b) of fed (closed symbols) and starved Pacific herring groups (open symbols) in the cold treatment (6.5°C). Symbol bars represent standard error. Significant differences ($p \leq 0.05$) between fed and starved groups for mass, protein, and R/D shown by ✦

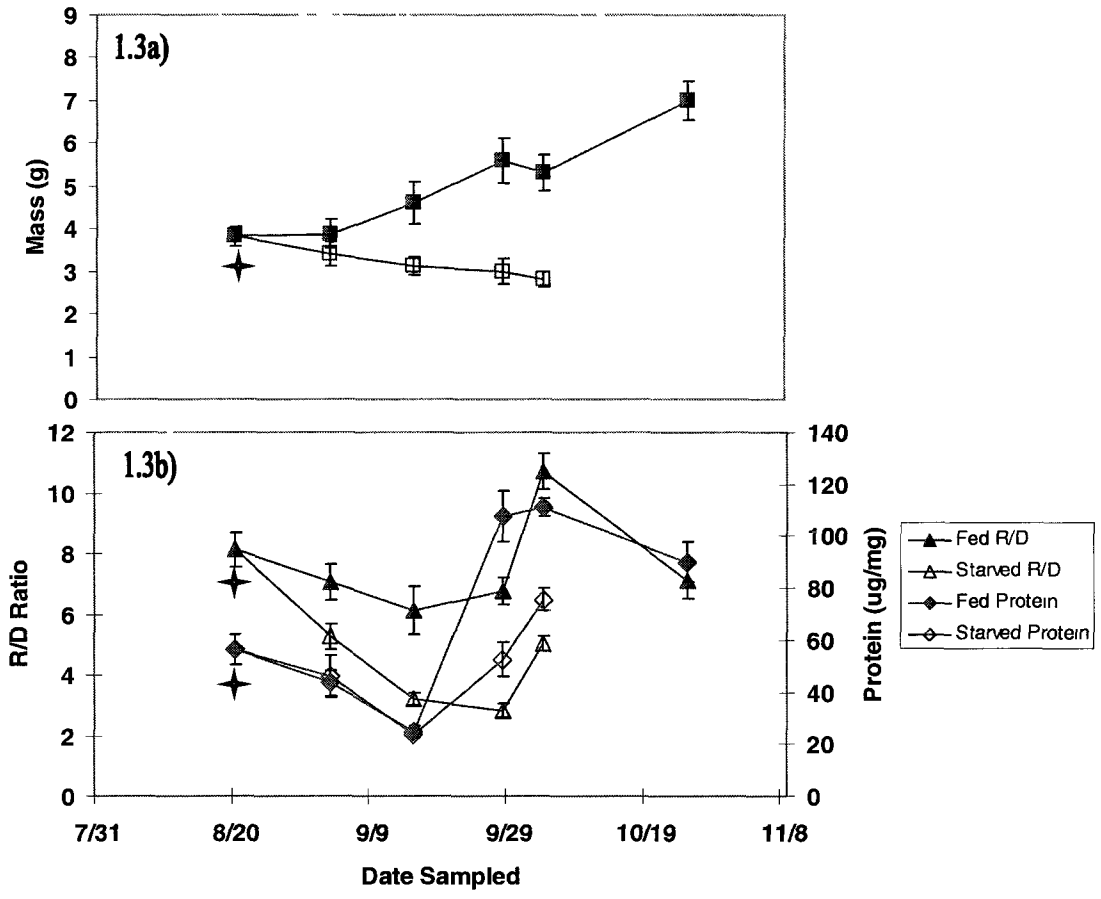

Fig. 1.3a,b: *Clupea pallasii*. Mass, protein, RNA/DNA in ambient treatment. Comparison between mass (1.3a), protein (1.3b), and RNA/DNA (R/D; 1.3b) of fed (closed symbols) and starved Pacific herring groups (open symbols) in the ambient treatment (8.5°C). Symbol bars represent standard error. Significant differences ($p \leq 0.05$) between fed and starved groups for mass, protein, and R/D shown by ✦

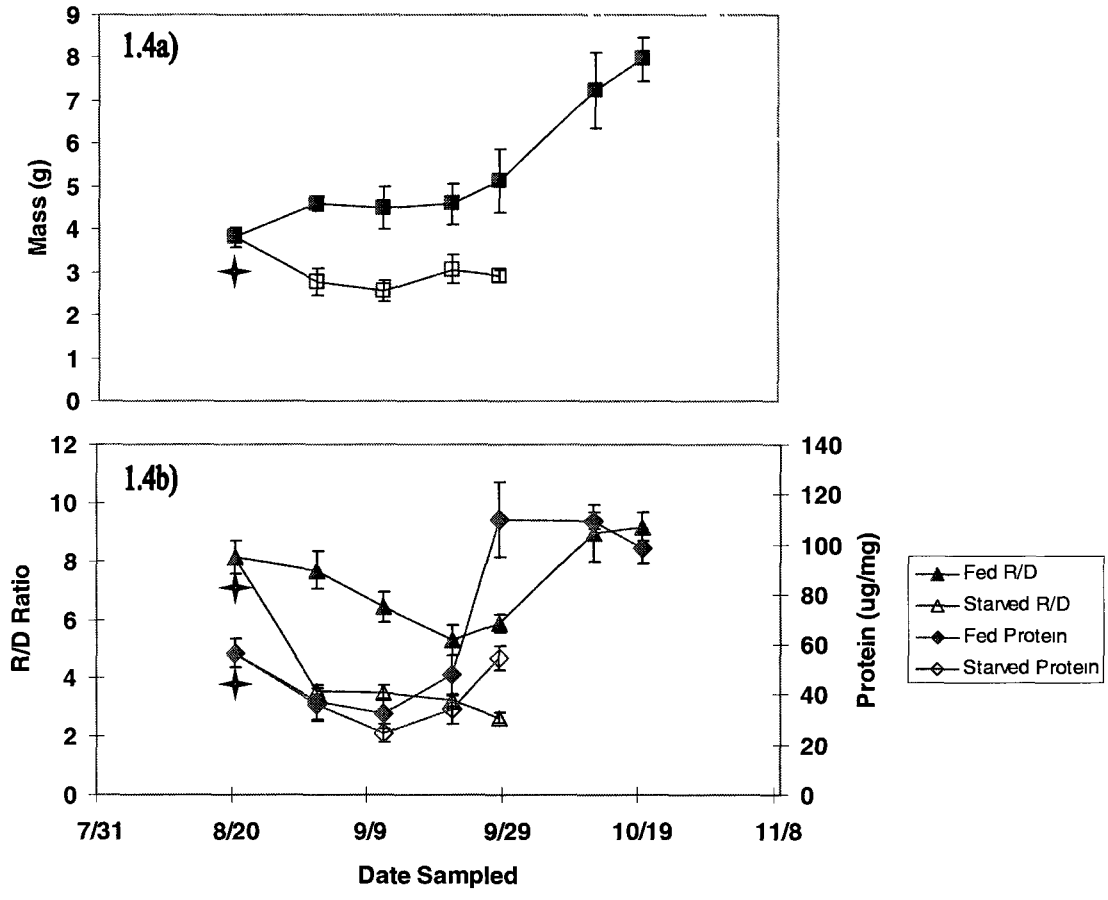

Fig. 1.4a,b: *Clupea pallasii*. Mass, protein, RNA/DNA in warm treatment. Comparison between mass (1.4a), protein (1.4b), and RNA/DNA (R/D; 1.4b) of fed (closed symbols) and starved Pacific herring groups (open symbols) in the hot treatment (12.5°C). Symbol bars represent standard error. Significant differences ($p \leq 0.05$) between fed and starved groups for mass, protein, and R/D shown by ✦

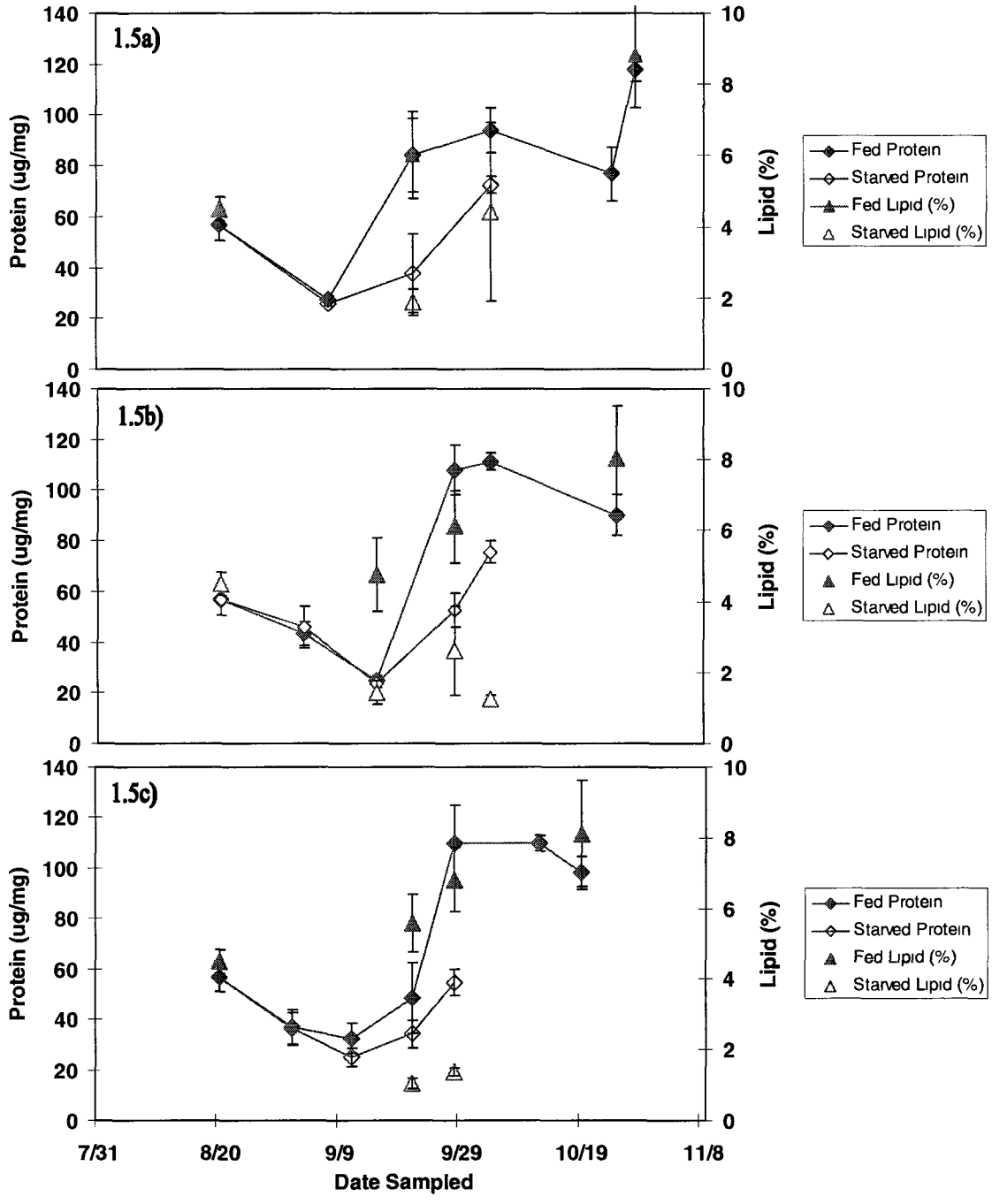

Fig. 1.5a,b,c: *Clupea pallasii.* Protein and total body lipid concentrations in fed and starved groups. Comparison of protein and total body lipid (%) between fed (closed symbols) and starved Pacific herring groups (open symbols) in the cold (6.5°C; fig.1.5a), ambient (8.5°C; fig.1.5b) and hot treatments (12.5°C; fig.1.5c). Symbol bars represent standard error.

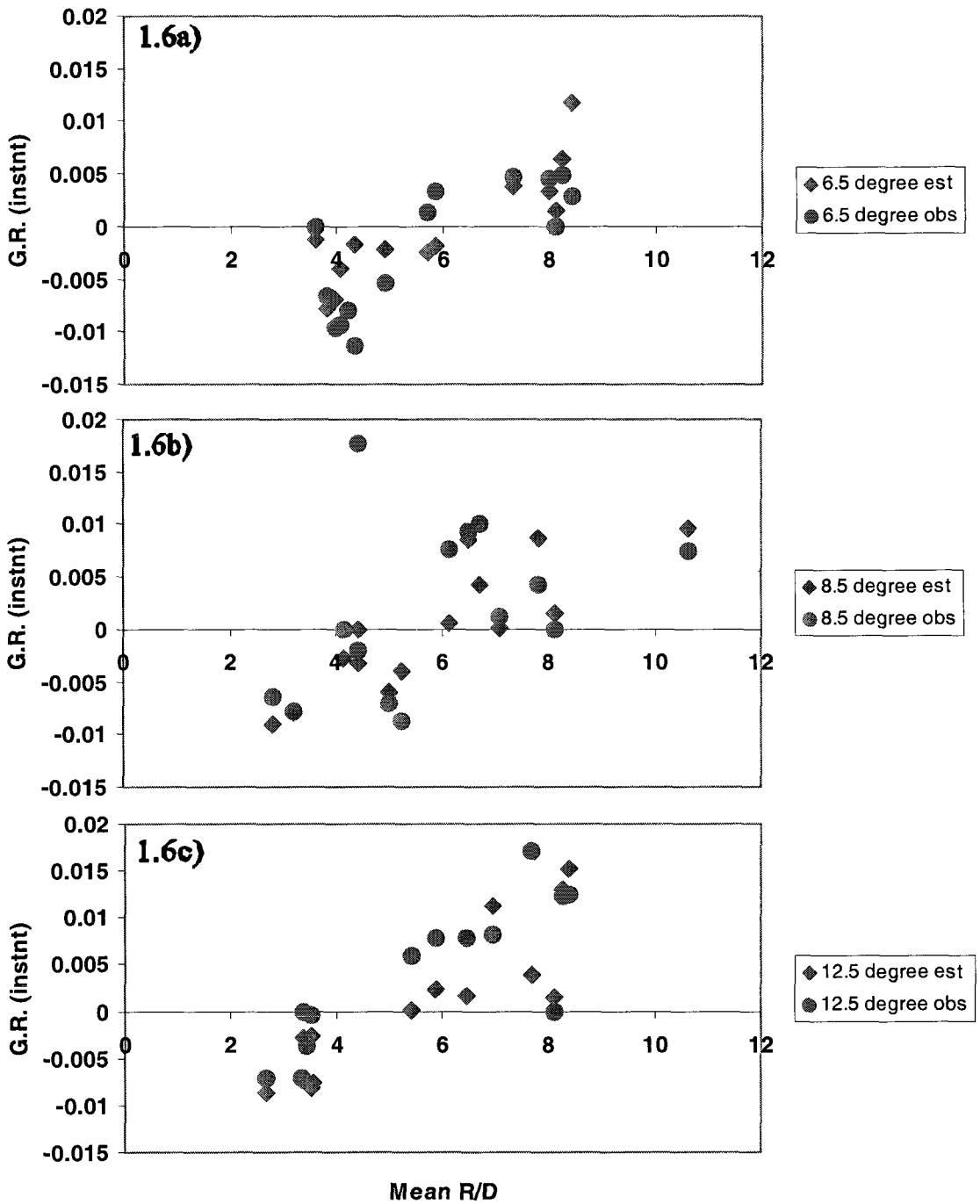

Fig 1.6a,b,c: *Clupea pallasii.* Observed and estimated growth rate comparisons. Comparison between observed (obs) and estimated (est) growth rates (from growth model) for Pacific herring in the cold (6.5°C; fig.1.6a), ambient (8.5°C; fig.1.6b), and hot treatments (12.5°C; fig.1.6c).

Table 1.1: *Clupea pallasii*. Growth in fed and starved/fed groups. Statistical comparison of body mass, fork length, protein concentration, and RNA/DNA between fed and starved/fed Pacific herring groups at the termination of the CG experiment across three treatments (6.5°C, 8.5°C, 12.5°C). *P*-values ≤ 0.05 are statistically significant.

	Comparison over all treatments	Comparison within temperature treatment between fed and starved/fed groups		
		6.5°C	8.5°C	12.5°C
Body Mass	< 0.001	0.4711	0.0867	0.0992
Length	0.004	0.9316	0.1939	0.3726
Protein	0.227	0.9037	0.4690	< 0.001
RNA/DNA	0.380	0.8836	0.8546	0.9320

Table 1.2: *Clupea pallasii*. Growth model regression. Multiple linear regression coefficients (+/-SE) describing the relationship between instantaneous growth rates (I.G.R.), mean RNA/DNA-a_1, mean mass (g)-b_1, and treatment temperature (°C)-c_1 for Pacific herring. Equation is of the form I.G.R.=a_1+b_1+c_1+C, where C is a constant. The regression was significant ($p < 0.001$). All parameters included in the model were significant ($p < 0.001$) except for temperature ($p = 0.062$). SE is standard error.

Coefficients (+/- SE)	a_1	b_1	c_1	C	Adjusted r^2
P. herring	0.00157 (0.0001)	0.00269 (0.0002)	0.000213 (0.0001)	-0.0233 (0.001)	0.618

Table 1.3: *Clupea pallasii*. AIC comparison. Model selection by Akaike Information Criterion (AIC) values. Coefficients (± SE) in alternative regression models of growth in juvenile Pacific herring. In all models, $p <$ 0.001. R/D = RNA/DNA ratio, T = treatment temperature, R/D*T = R/D and temperature interaction, K = number of model parameters (including intercept), Δ AIC = difference in AIC with respect to the best fit model.

#	Model Parameters						Intercept	r^2	K	AIC	Δ AIC
	R/D	Mean R/D	Body Mass	Mean Body Mass	T	R/D*T					
1		0.0015 (0.0002)		0.0026 (0.0002)	0.0002 (0.0001)		-0.0233	0.618	4	-1948	0
2		0.0024 (0.0001)	0.0010 (0.0001)		0.0004 (0.0001)		-0.0228	0.548	4	-1918	30
3	-0.0007 (0.0005)		0.0014 (0.0001)		-0.0008 (0.0003)	0.0002 (0.00005)	-0.0052	0.428	5	-1872	76
4	0.0005 (0.0002)		0.0014 (0.0001)			0.00008 (0.00002)	-0.0139	0.419	4	-1870	78
5	0.0012 (0.0001)		0.0015 (0.0001)		0.0003 (0.0001)		-0.0170	0.407	4	-1867	81
6	0.0007 (0.0002)					0.0001 (0.00002)	-0.0107	0.331	3	-1845	103
7	0.0019 (0.0001)				0.0005 (0.0001)		-0.0158	0.306	3	-1838	110

Chapter 2: The Effect of Temperature and Nutrition on RNA/DNA Ratio and Growth of Pacific Cod (*Gadus macrocephalus*) and Walleye Pollock (*Theragra chalcogramma*) Larvae [1]

Abstract:

Pacific cod (*Gadus macrocephalus*) and walleye pollock (*Theragra chalcogramma*) are among the most economically and ecologically important groundfish species in the North Pacific Ocean. In spite of their importance, little is known about many aspects of their physiology, specifically about larval growth strategies in these fish. Since larval fish growth may determine future growth patterns, which could affect recruitment success, assessments of larval growth strategies might improve predictive growth models. In this study, nucleic acids (RNA and DNA) were used to index early growth in yolk-sac Pacific cod and walleye pollock larvae cultured at two temperatures (5°C and 8°C) and in yolk-sac Pacific cod cultured in two nutritional states (fed and starved). Growth corresponded to changes in RNA/DNA. Growth responses in Pacific cod and walleye pollock larvae were affected by small differences in temperature. Exposure to the lower temperature resulted in higher RNA/DNA in both Pacific cod and walleye pollock larvae. Based on nucleic acid patterns during larval development, it was possible to identify distinct growth stanzas in Pacific cod larvae.

[1] Ashwin Sreenivasan, Ronald A. Heintz, Stanley D. Rice, Thomas P. Hurst, Benjamin J. Laurel. The Effect of Temperature and Nutrition on RNA/DNA ratio and Growth of Pacific Cod (*Gadus macrocephalus*) and Walleye Pollock (*Theragra chalcogramma*) Larvae. Prepared for submission in Marine Ecology Progress Series

Introduction

Pacific cod (*Gadus macrocephalus*) and walleye pollock (*Theragra chalcogramma*) are among the most numerous (Mecklenburg et al. 2002) and commercially important groundfish species in the North Pacific Ocean (Jewett 1978, Grant et al. 1987, Stepanenko 1995, Beamish et al. 2004, Bacheler et al. 2010, Hiatt et al. 2010). Distributed from southern California north to the Bering Sea, the Aleutian Islands, the Gulf of Anadyr and the Kurile Islands, the Okhotsk Sea, the Yellow Sea, and the Sea of Japan (Ketchen 1961, Bakkala 1984, Bakkala et al. 1984), Pacific cod are an important upper-trophic level species in subarctic ecosystems (Sakurai and Hattori 1996). Walleye pollock also are broadly distributed from the Bering Sea and the Gulf of Alaska to Japan (Bacheler et al. 2010).

Assessments of early larval growth and development are, however, limited for both species. Estimating larval growth can be important since growth during this stage could determine future growth (Jonsson et al. 2005, Koedijk et al. 2010 *a*, *b*). Fish larvae are especially sensitive to minor changes in ecological factors, including temperature and nutrition (Takatsu et al. 1995), which affect growth. Most growth and recruitment assessments in nature have been feasible only after fish have reached the juvenile or adult stages. Applying most growth indices to individual larvae is not feasible because they lack adequate tissue mass. These limitations are intensified by the fragility of larvae, which makes capture of viable samples difficult, and by associated problems of sample storage and tissue shrinkage.

The early development, behavior, and physiology of larval Pacific cod and walleye pollock have been examined in a few studies (Alderdice and Forrester 1971, Sogard and Olla 1996, Theilacker et al. 1996, Laurel et al. 2008, Hurst et al. 2010, Laurel et al. 2010, Laurel et al. 2011). These studies have highlighted the complexities of larval behavior and physiology, especially in the early post-hatch period. Diel migratory behavior in larval Pacific cod can be determined by a combination of factors, including development stage, temperature, and photoperiod (Hurst et al. 2009). Growth in turn can be affected by interacting factors, including prey availability, temperature, and development stage (Laurel et al. 2011).

In light of the studies carried out so far, a physiological index of larval growth coupled with energetic assessments in predictive growth models can improve our understanding of natural variation of larval survival and of recruitment. This laboratory study investigated whole-body nucleic acid concentrations as an index of growth in yolk-sac Pacific cod and walleye pollock larvae, assessing the responses of growth and nucleic acids to different temperatures and nutritional states.

Larval growth of fish is affected by physiological and morphological changes associated with temperature and nutrition (Blaxter 1992, Pavlov et al. 1994, De March 1995, Kamler 2002, Gabillard et al. 2003*a, b,* Amberg et al. 2008, Kamler 2008, Li and Leatherland 2008, Janhunen et al. 2010, Teletchea and Fontaine 2010). Protein synthesis underlying larval growth is dependent on RNA concentration (translational capacity) and RNA-specific protein synthesis rate (translational efficiency; McMillan and Houlihan 1988, Fraser and Rogers 2007), and the latter is affected by temperature. A change in the

relationship between translational efficiency/capacity and growth can affect energy budgets, an important consequence for larvae since they lack energetic reserves (Theilacker et al. 1996).

Even though assessing larval growth can be problematic, nucleic acid (R/D) ratios can be used as a sensitive growth index in fish larvae (Buckley 1979, 1984, Robinson and Ware 1988, McLaughlin et al. 1995, Buckley et al. 1999, Weber et al. 2003, Caldarone 2005, Stierhoff et al. 2009). While DNA concentrations remain stable (Buckley 1984, McLaughlin et al. 1995), cellular RNA concentrations increase or decrease along with protein synthesis, indicating growth and nutritional condition (Weber et al. 2003). However, temperature affects RNA translational efficiency (Lewis and Driedzic 2007) changing the ratio-growth relationship. This prevents direct R/D comparisons between fish observed at temperature differences >2°C (Buckley et al. 1999), and requires calibration of nucleic acid concentrations with growth rates over a temperature range, that is R/D-Growth-Temperature calibrated models (Buckley 1984). Nucleic acid ratios could also be developed as indicators of catabolic stages in larvae approaching terminal starvation, i.e. a "baseline" ratio.

We used R/D ratios to observe the effects of temperature on growth in Pacific cod and walleye pollock larvae, and to measure the effects of different nutritional states (fed and starved) on growth in Pacific cod larvae. The aim of measuring R/D ratios was to estimate larval energetic and growth parameters and incorporate those parameters in a predictive growth model. Our objectives were to a) examine nucleic acid and early growth responses in Pacific cod and walleye pollock larvae at different temperatures, b)

examine nucleic acid and growth responses in Pacific cod larvae at different nutritional states, and c) build a predictive larval Pacific cod growth model based on nucleic acids measured over a range of temperatures and different nutritional states.

Materials and Methods

This study included two temperature treatment experiments and a starvation experiment. In the temperature treatment experiments, Pacific cod and walleye pollock larvae were fed and cultured at two temperatures; in the starvation experiment, groups of Pacific cod larvae were either fed or starved at a single temperature.

Pacific cod larvae used in both experiments were hatched and cultured in the National Oceanic and Atmospheric Administration (NOAA) Alaska Fisheries Science Center (AFSC) Laboratory, Newport, Oregon in April 2008 and May 2009. Eggs were obtained from adults at their spawning grounds in Chiniak Bay, Kodiak Island, Alaska. All mixed gametes were held in incubation trays at 4°C, after which fertilized eggs (24 hours post-fertilization) were shipped in insulated containers to the AFSC. Eggs were held in flow-through plastic trays (~4 liter capacity) and maintained at 4°C until hatch, approximately 19-22 days post-fertilization.

Walleye pollock eggs were collected from mature pollock broodstock maintained by the AFSC at the Hatfield Marine Science Center. Eggs were collected using nylon mesh collectors placed on the outflow of broodstock tanks. Larvae hatched in April 2008.

Pacific Cod Temperature Experiment

After hatching, larvae (n = 120) were immediately sampled to measure initial condition. Larvae were then randomly assigned to two temperature treatments: cool (5°C) and warm (8°C). Larvae at 0 days post hatch (dph) were in the size range ~4.4-5 mm total length. In each treatment, larvae were held in three tanks (100 liter capacity; 400 larvae each), with a total of six tanks. All tanks had a flow rate of ~250 mL minute^{-1} seawater and were initially held at 4°C, after which water temperatures were adjusted to experimental conditions over 48 hours. Larvae were held at a 12:12 hour light:dark photoperiod regime (Hurst et al. 2010).

Larvae in both treatments were fed on a combined diet of rotifers (*Brachionus plicatilis*; twice daily) and microparticulate dry food (Otohime A, Marubeni Nisshin Feed Co., Tokyo; two-three times daily). After the initial sampling after hatch, larvae were sampled (10 larvae/tank/treatment) at 23 and 36 dph. At each sampling period, larvae were removed from tanks using droppers, placed in individual 1500 µL microcentrifuge vials on ice, and then stored at -80°C until analysis. All sampled larvae were individually photographed before being placed in vials, to measure total length post-sampling using imaging software. Larval fish lengths were measured to the nearest 0.005mm using NIS Elements D, Nikon Instruments, Melville, NY.

Growth rates (length-GR_L) were calculated for larvae in both treatments from each sampling date (t_2) to the hatch date (t_1) using the formula:

$$GR_L = (\ln l_{t2} - \ln l_{t1}) / (t_2 - t_1)$$

where l = mean length (mm) and ln = natural logarithm.

Pacific Cod Starvation Experiment

Larvae were initially held at 6.5°C in two common tanks (100 liter capacity) with a flow rate of ~250 mL minute^{-1} seawater, before transfer to fed and starved treatment tanks (two tanks/treatment; 40 liter capacity) held at 6.5°C. All larvae at 0 dph were in the size range ~5-6.5 mm total length. The feeding regimen was similar to the temperature experiment.

During May to June 2009 larvae were sampled across three sampling periods initiated at 0, 9, and 20 dph. The sampling protocol was similar to the temperature experiment. At the start of each sampling, larvae were sampled from the common tanks (10 larvae/tank), and randomly assigned to the fed or starved treatment tanks (60 larvae/tank). Larvae in the starved treatment tanks were sampled (10 larvae /tank) when the population in the tanks reached 10% mortality and 50% mortality, with corresponding sampling in the fed treatment. Individual larvae were photographed to measure total length post-sampling using imaging software. The experiment was terminated at 25 dph.

Larval fish lengths were measured to the nearest 0.005mm using Image-Pro Plus, Media Cybernetics, Bethesda, MD. Growth rates (length-GR_L) were calculated similar to the temperature experiment.

Walleye Pollock Temperature Experiment

Larvae were cultured in duplicate tanks (n = 200 larvae/tank) at a cool (5°C) and warm (8°C) temperature treatment. All larvae were in the size range ~6-8 mm length and were fed for the duration of the experiment. All experimental conditions and sampling protocols were identical to the Pacific cod temperature experiment, except for the time of

sampling (0, 19, and 40 dph). At 0 dph, larvae were sampled from each tank (15 larvae/tank/treatment), followed by sampling at 19 and 40 dph (10 larvae/tank/treatment). Growth rates (length-GR_L) were calculated similar to the Pacific cod temperature experiment.

Biochemical Analyses

Nucleic acid ratios in both Pacific cod experiments and in the walleye pollock experiment were measured under a one dye-one enzyme (RNase) fluorometric protocol modified from Caldarone et al. (2001). Whole larvae were processed directly in their individual storage vials thus avoiding any tissue loss. Initially, 150 µL 1% sarcosil Tris-EDTA buffer was added to each sample vial. All sample vials were then vortexed for 60 minutes. Samples were further diluted with 1350 µL Tris-EDTA buffer, and centrifuged for 15 minutes at 14000*g* in a Sorvall Legend Micro 21 R refrigerated centrifuge (Thermo Scientific). Supernatants were then treated with 75µL ethidium bromide (5µg ml^{-1}) according to the protocol outlined by Caldarone et al. (2001). Total fluorescence was measured in a Wallac 1420 microplate spectrophotometer (Perkin Elmer, Waltham, MA) at excitation and emission wavelengths of 355nm and 600nm respectively. Samples were treated with RNase, and the resulting reduced fluorescence measured to obtain DNA fluorescence; RNA fluorescence was obtained through subtraction of DNA fluorescence from the total fluorescence. Calibration curves were constructed using serial dilutions of 18s-28s rRNA (Sigma R-0889) and calf thymus DNA (Sigma D-4764) standards. Supernatants for R/D were read on Corning NBS 96-well black flat-bottom microplates (75µL samples). Every R/D sample was run in three individual wells. Fluorescence

values for all three wells were read four times, and the coefficient of variation associated with four reads was examined. When the variation was higher than 10%, the individual well fluorescence values were examined, and the data point causing the high coefficient of variation was excluded from the analysis.

Statistical Analyses:

In all analyses, differences between groups were considered significant if $p \leq 0.05$

Pacific Cod Temperature Experiment

Growth was compared between larvae at 5°C and 8°C. A General Linear Model (GLM) with Tukey's posthoc test was used to compare length in larvae between treatments over 0-36 dph, with length as the dependent variable, and temperature treatment and sampling day as independent variables. A GLM was used to compare instantaneous growth rates (IGR) in larvae between treatments, with IGR as the dependent variable, and temperature treatment and sampling day as independent variables. A GLM with Tukey's test was used to compare R/D of larvae between treatments, with R/D as the dependent variable, and temperature treatment and sampling day as independent variables. All statistical analyses were made with Minitab v. 14 (Minitab Inc., State College, Pennsylvania).

Pacific Cod Starvation Experiment

Growth was compared between larvae in fed and starved treatments. A GLM was used to compare IGR of fed and starved larvae, with growth rates as the dependent variable, and treatment as well as sampling day as predictor variables. A GLM with

Tukey's test was used to compare R/D of fed and starved larvae over 0-25 dph, with R/D as the dependent variable, and sampling day and treatment as the independent variables

The R/D of starved larvae between 10% and 50% mortality was compared within each sampling period using two sample T-tests. The R/D of starved larvae at both 10% and 50% mortality was compared between the last two sampling periods with a two sample T-test.

A GLM was used to compare individual nucleic acids of fed and starved larvae over 0-25 dph, with RNA or DNA as the dependent variable, and sampling day and treatment as independent variables. All statistical analyses were made with Minitab v. 14 (Minitab Inc., State College, Pennsylvania).

Pacific Cod Growth Model

Calibrated growth models were generated through multiple linear regression, describing the relationship between instantaneous growth rates (IGR) and variables including R/D, RNA, DNA, and temperature. A second-order Akaike's information criterion (AICc) was used to select the best fit model from the model data set (Wagenmakers and Farrell 2004). Estimated growth rates were compared with observed growth rates for all temperature treatments using a two-sample T-test. The regression models were generated by Minitab v. 14 (Minitab Inc., State College, Pennsylvania).

Depending on the model, R/D ratios or RNA and DNA from both Pacific cod experiments were combined for the individual growth models in the model data set. Mean tank values for each sampling day (R/D, RNA, DNA) were log-transformed. The growth model only included data from larvae 23 and 36 dph in the temperature

experiment and from 9 dph onward (fed fish only) in the terminal starvation experiment. This was due to lack of a correlation between R/D and growth in the early larval stages, possibly due to presence of pre-existing yolk protein.

Walleye Pollock Temperature Experiment

Growth was compared between larvae at 5°C and 8°C. All statistical analyses were identical to the Pacific cod temperature experiment and were made with Minitab v. 14 (Minitab Inc., State College, Pennsylvania).

Results

Pacific Cod Temperature Experiment

Larvae at 8°C were longer than larvae at 5°C at the end of the experiment ($p < 0.001$; Fig 2.1a) with a difference in length between treatments at 23 dph and 36 dph ($p < 0.001$; Table 2.1). Larvae at 8°C grew faster than larvae at 5°C and had significantly higher instantaneous growth rates over the sampling period ($p < 0.001$; Table 2.1). Larvae at 5°C had higher R/D than larvae at 8°C at the end of the experiment (Fig. 2.1b), with a difference between temperature treatments at 36 dph ($p < 0.001$; Table 2.1).

Pacific Cod Starvation Experiment

Fed larvae had significantly higher instantaneous growth rates ($p < 0.001$) over 5-25 dph relative to starved larvae (Fig. 2.2a; Table 2.2). Nucleic acid ratios were higher in fed larvae relative to starved larvae over 0-25 dph ($p < 0.001$; Fig. 2.2b); there were differences in nucleic acid ratios between fed and starved larvae at 5 dph ($p = 0.0061$), 11

dph ($p = 0.0101$), 15 dph ($p = 0.0003$), 23 dph ($p = 0.0180$), and 25 dph ($p = 0.0458$; Table 2.2).

A comparison of R/D between 10% and 50% mortality in starved larvae showed differences over 11-15 dph ($p = 0.034$) but not over 2-5 dph ($p = 0.134$) or 23-25 dph ($p = 0.798$). Comparison of R/D at 10% mortality between the second and third sampling periods (11 and 23 dph) showed no difference ($p = 0.071$). However, R/D differed at 50% mortality ($p = 0.006$) between second and third sampling periods (15 and 25 dph).

The RNA and DNA concentrations were higher in fed larvae relative to starved larvae over 0-25 dph ($p < 0.001$). The RNA concentrations in the fed larvae remained steady until 9 dph, increasing slightly by 11 dph (Fig. 2.3b). From 11-20 dph RNA concentrations remained steady, and increased after 20 dph. In contrast, DNA concentrations in both fed and starved larvae increased until 11 dph (Fig. 2.3c). From 11-20 dph, DNA concentrations in fed larvae remained steady, and increased after 20 dph.

Pacific Cod Growth Model Synthesis

A significant positive relationship was found between IGR, log RNA, log DNA, and treatment temperature ($p < 0.001$; Table 2.3). This growth model was chosen from the model data set based on a comparison of AICc values (AICc = -122; Table 2.4), and incorporated IGR, log RNA, log DNA, and treatment temperature in a multiple linear regression. The response variable was IGR; log RNA, log DNA, and treatment temperature were predictor variables. Estimated and observed growth rates did not differ for fish at 5°C ($p = 0.0180$), 6.5°C ($p = 0.479$), and 8°C ($p = 0.259$)

Walleye Pollock Temperature Experiment

Larvae at 8°C were longer than larvae at 5°C at the end of the experiment ($p < 0.001$; Fig. 2.4a), with a difference between treatments at 40 dph ($p < 0.001$; Table 2.5). Larvae at 8°C grew faster than larvae at 5°C and had significantly higher instantaneous growth rates over the sampling period ($p < 0.001$; Table 2.5). Larvae at 5°C had higher R/D than larvae at 8°C at the end of the experiment ($p < 0.001$; Fig 2.4b), with a difference between treatments at 0 dph ($p = 0.0050$) and 40 dph ($p < 0.001$; Table 2.5).

Discussion

Lower R/D coupled with higher growth in larvae at 8°C indicated that translational efficiency defines early growth in Pacific cod and walleye pollock. Early larval fish growth is predominantly due to protein synthesis and deposition (Tong et al. 2010), determined by RNA-specific protein synthesis rate (translational efficiency) and RNA concentration (translational capacity; Smith and Ottema 2006, Fraser and Rogers 2007). The relative effects of translational efficiency and capacity on growth can be influenced by temperature (Foster et al. 1992, Treberg et al. 2005). Translational efficiency also defines early larval growth in Atlantic herring (*Clupea harengus*; Houlihan et al. 1995), African catfish (*Clarias gariepinus*; Smith and Ottema 2006), and rainbow trout (*Oncorhynchus mykiss*; Peragon et al. 2001).

Growth in larval Pacific cod and walleye pollock is affected by small differences in temperature. Exposure to lower temperature reduced translational efficiency in both gadids, resulting in growth driven predominantly by translational capacity. This was suggested by a lack of correspondence between R/D and growth rates. As expected,

larvae at 8°C had higher growth rates and were longer than larvae at 5°C. This higher growth, coupled with lower R/D relative to larvae at 5°C, suggested heightened translational efficiency. The higher R/D at 5°C suggests a compensatory mechanism to maintain protein synthesis, i.e., heightened translational capacity in lieu of reduced translational efficiency. But with the high costs of RNA and protein synthesis (Bocharova et al. 1992, Houlihan et al. 1995, Smith and Ottema 2006), regardless of cost mitigation strategies (Houlihan et al. 1988, Wieser 1995, Smith et al. 2000, Smith and Ottema 2006), higher translational efficiency is energetically advantageous. The effect of this trade-off between translational efficiency/capacity could be significant as larvae grow and increase in size. Another potential reason for higher R/D at 5°C could be mismatched RNA degradation and synthesis rates, due to a temperature effect on enzymes essential to these processes. In this case, higher R/D would not be an active compensatory measure corresponding to heightened protein synthesis. While protein synthesis rates were not measured in this experiment, Atlantic cod exposed to colder temperatures showed heightened translational capacity active in maintaining protein synthesis (Foster et al. 1992, Treberg et al. 2005), suggesting that the higher R/D in this experiment was a compensatory strategy.

The trade-off between translational efficiency/capacity can be examined in terms of the R/D divergence between larvae. The timing of the divergence suggested heightened energetic demands as larvae aged, possibly due to growth and increased protein synthesis, as larvae increased in size and structural complexity. Crucially, the consistently lower growth at 5°C suggested lower translational efficiency prior to the

divergence in R/D ratios. However, the lack of an immediate compensatory response could be due to energetic costs involved in heightened translational capacity as well as lower larval energy requirements. As larvae grow, energetic constraints could lead to heightened translational capacity. However, this could be energetically expensive, potentially affecting long-term growth as in Atlantic cod larvae (Koedijk et al. 2010*a, b*).

This experiment highlighted early larval Pacific cod and walleye pollock growth strategies in terms of translational efficiency and capacity. Pacific cod and walleye pollock larvae appear to have physiological compensatory mechanisms to maintain growth trajectories, which could be energetically expensive. Cellular growth indices such as R/D can highlight physiological growth strategies, a prerequisite to further studying any associated metabolic costs which should be accounted for in growth assessments.

The terminal fasting experiment examined the growth and R/D relationship in Pacific cod larvae (0-25 dph) based on nutritional state. In contrast to other vertebrates, where post-natal growth is hypertrophic (an increase in muscle fiber size; Smith et al. 2000) teleost growth is a combination of hypertrophy and hyperplasia (formation of new muscle fibers). Early teleost development involves hyperplasic organogenesis (Tanaka et al. 1996, Zouiten et al. 2008, Tong et al. 2010) followed by somatic growth which is both hyperplasic and hypertrophic (Westerman and Holt 1994, Tong et al. 2010). These development patterns are common across species, shown by a steady decrease in R/D ratios over the immediate post-hatch period (Clemmeson 1987, Puvanendran and Brown 1999, Caldarone et al. 2003).

In common with other fish species, R/D in Pacific cod larvae decreased over the initial post-hatch period, and then stabilized. The initial decrease was due to increasing DNA in fed and starved larvae (0-11 dph) coupled with stable (fed group) or reduced (starved group) RNA. The increased DNA content with stable or reduced RNA could correspond to hyperplasic organogenesis. Both RNA and DNA in fed larvae stabilized over 11-20 dph, potentially the transition phase after yolk absorption to complete reliance on exogenous food. Stable R/D in fed larvae implied a lack of growth during the transition to external food, evidenced by a corresponding decrease in growth rate. A similar trend was observed in larval Turbot (*Scophthalmus maximus*) after yolk depletion (Tong et al. 2010). Laurel et al. (2008) found that Pacific cod larvae had no growth immediately after yolk absorption, while fed Atlantic cod larvae also had the lowest growth and R/D coinciding with yolk absorption (Caldarone et al. 2003). Atlantic cod larvae have also shown closely corresponding transition periods (Yin and Blaxter 1986, Hunt von Herbing et al. 1996, Caldarone et al. 2003) at similar temperatures. While the rate of yolk absorption can be temperature dependent (Laurel et al. 2008), the timing of the transition phase (11-20 dph) integrates the initial increase in DNA (0-11 dph) with yolk absorption.

The timing of the transition phase suggested yolk absorption over the preceding 0-11 dph, coinciding with the increase in DNA potentially indicating organogenesis. Since organogenesis can utilize lipid and protein components of yolk (Cejas et al. 2004), yolk in Pacific cod larvae appears primarily utilized in organogenesis. While yolk is the most cost-effective source of protein, the energetic cost of protein absorption remains

substantial (Smith and Ottema 2006). However, with even higher costs of protein synthesis (Hawkins 1991, Smith and Houlihan 1995), efficient larval growth strategies emphasize absorption of preexisting yolk protein into tissues (Wieser et al. 1988). Similar growth strategies have been observed in larval African catfish (*Clarias gariepinus*; Smith and Ottema 2006), Atlantic herring (*Clupea harengus*; Houlihan et al. 1995), and nase (*Chondrostoma nasus*; Houlihan et al. 1992). Yolk absorption coinciding with organogenesis was observed in both starved and fed treatments. This suggested the relative importance of organogenesis, as starved larvae did not divert any yolk towards somatic growth, and had relatively lower growth rates, R/D, and RNA.

In conjunction with increases in R/D and RNA after the transition period (20 dph), the growth rate in fed larvae increased. This indicated the channeling of resources to somatic growth after organogenesis. In larval Pacific cod, increased growth after the transition phase also coincided with the start of diel vertical migrations (Hurst et al. 2009) and heightened responses to prey (Colton and Hurst 2010). In common with other fish species, this growth phase, characterized by increasing RNA, DNA, R/D, and growth rate, is probably a combination of hypertrophy and hyperplasia (Tong et al. 2010).

Nucleic acid ratios of starved larvae could potentially be used as indicators of terminal starvation, i.e., a "baseline" ratio. Averages of ratios from larvae sampled at 10% and 50% starvation over the 2nd and 3rd sampling periods, while preliminary and approximate (~2.5), have promise as a starvation index, but require further validation studies.

The Pacific cod growth model incorporated nucleic acids (RNA and DNA) and temperature as variables in predicting growth rate. This model estimated 65.5% of the observed variation in growth rate. Based on both adjusted r^2 and AIC_C values, individual nucleic acids appear to predict early larval growth in Pacific cod better than a model based on R/D ratios. With the lack of correlation between growth and R/D in early stage yolk-sac larvae (Caldarone et al. 2003), this growth model did not include data from 0 dph larvae (temperature experiment) or from 0-5 dph larvae (terminal starvation experiment). The ready availability of protein (yolk) in early larval stages could cause the lack of correlation between nucleic acids and growth. This growth model relates nucleic acids to growth and condition of individual larvae and can have applications in management, since growth and condition estimates are required in recruitment and stock assessments. This model is useful in estimating growth in Pacific cod at an early life-stage. Additionally, it enables growth estimation in field-sampled larvae using a single measure, a requirement since it is not realistically possible to periodically sample the same fish in the field to estimate growth.

Conclusion

Nucleic acid ratios have potential as a growth index in larval Pacific cod and walleye pollock; this index was sensitive enough to bring out differences in early larval growth responses and physiological strategies. Larval Pacific cod and walleye pollock growth strategies are affected by small differences in temperature, while nutritional state also affected early growth in Pacific cod. Exposure to a lower temperature resulted in higher R/D in both cod and pollock larvae, potentially a compensatory strategy. This

could be due to increased energetic demands as larvae increase in size and complexity. These higher ratios were only manifested after a period of consistently lower growth, suggesting costs associated with this strategy.

Fed Pacific cod larvae as expected had higher growth relative to starved larvae, shown by R/D and growth rates. Changes in R/D, RNA, and DNA could be used to identify distinct stanzas in early growth of Pacific cod larvae, possibly yolk absorption and organogenesis, exogenous feeding (transition phase), and post-transition growth. Similar growth stages are seen in larvae of other fish species. Yolk appears predominantly utilized towards organogenesis, even in starved fish. While R/D reduced in both treatments over the yolk absorption phase, the R/D in the starved treatment remained lower, indicating that exogenous food supply may be added to energy reserves available to growth despite the presence of yolk. The Pacific cod growth model was able to estimate 65.5% of observed variation in growth rate. This model could have applications in management, since growth and condition estimates are required for recruitment and stock assessments.

Acknowledgements

We thank Elaine Caldarone, who was extremely generous with her time in helping to adapt her R/D protocol to this research, as well as Dr. Franz Mueter, University of Alaska Fairbanks, and Dr. Chris Hay-Jahans, University of Alaska Southeast, for their advice on statistical methods. We also thank Dr. Sherry Tamone, University of Alaska Southeast, the Rasmuson Fisheries Foundation for fellowship support awarded to AS, and NOAA, who supported this research.

Literature Cited

Alderdice DF, Forrester CR (1971) Effects of salinity, temperature, and dissolved oxygen on early development of the Pacific cod (*Gadus macrocephalus*). J Fish Res Board Can 28: 883-902.

Amberg JJ, Myr C, Kamisaka Y, Jordal A.-E.O, Rust MB, Hardy RW, Koedijk R, Ronnestad I (2008) Expression of the oligopeptide transporter, PepT1, in larval Atlantic cod (*Gadus morhua*). Comp Biochem Physiol B 150: 177-182.

Bacheler NM, Ciannelli L, Bailey KM, Duffy-Anderson JT (2010) Spatial and temporal patterns of walleye pollock (*Theragra chalcogramma*) spawning in the eastern Bering Sea inferred from egg and larval distributions. Fish Oceanogr 19: 107-120.

Bakkala RG (1984) Pacific cod of the eastern Bering Sea. Bull Int North Pac Fish Comm 42: 157-179.

Bakkala RG, Mishima S, Westrheim SJ, Zhang CI, Brown ES (1984) Distribution of Pacific cod (*Gadus macrocephalus*) in the North Pacific Ocean. Bull Int North Pac Fish Comm 42: 111-115.

Beamish RJ, Benson AJ, Sweeting RM, Neville CM (2004) Regimes and the history of the major fisheries off Canada's west coast. Prog Oceanogr 60: 355-385.

Blaxter JHS (1992) The effect of temperature on larval fishes. Neth J Zool 42: 336-357.

Bocharova LS, Gordon RY, Arkhipov VI (1992) Uridine uptake and RNA synthesis in the brain of torpid and awakened ground squirrels. Comp Biochem Physiol B 101: 189-192.

Buckley LJ (1979) Relationships between RNA-DNA ratio, prey density, and growth rate in Atlantic cod (*Gadus morhua*) larvae. J Fish Res Board Can 36: 1497-1502.

Buckley LJ (1984) RNA-DNA ratio: an index of larval fish growth in the sea. Mar Biol 80: 291-298.

Buckley L, Caldarone E, Ong T.-L (1999) RNA–DNA ratio and other nucleic acid-based indicators for growth and condition of marine fishes. Hydrobiologia 401: 265-277.

Caldarone EM (2005) Estimating growth in haddock larvae *Melanogrammus aeglefinus* from RNA:DNA ratios and water temperature. Mar Ecol Prog Ser 293: 241-252.

Caldarone EM, St. Onge-Burns JM, Buckley LJ (2001) Protocol and guide for estimating nucleic acids in larval fish using a fluorescence microplate reader. Northeast Fisheries Science Center Reference Document 01-11.

Caldarone EM, St. Onge-Burns JM, Buckley LJ (2003) Relationship of RNA/DNA ratio and temperature to growth in larvae of Atlantic cod *Gadus morhua.* Mar Ecol Prog Ser 262: 229-240.

Cejas JR, Almansa E, Jerez S, Bolanos A, Felipe B, Lorenzo A (2004) Changes in lipid class and fatty acid composition during development in white seabream (*Diplodus sargus*) eggs and larvae. Comp Biochem Physiol B: 139: 209-216.

Clemmesen CM (1987) Laboratory studies on RNA/DNA ratios of starved and fed herring (*Clupea harengus*) and turbot (*Scophthalmus maximus*) larvae. J Cons int Explor Mer 43: 122-128.

Colton AR, Hurst TP (2010) Behavioral responses to light gradients, olfactory cues, and prey in larvae of two North Pacific gadids (*Gadus macrocephalus* and *Theragra chalcogramma*). Environ Biol Fish 88: 39-49.

DeMarch BGE (1995) Effects of incubation temperature on the hatching success of Arctic char eggs. Prog Fish-Cult 57: 132-136.

Foster AR, Houlihan DF, Hall SJ, Burren LJ (1992) The effect of temperature acclimation on protein synthesis rates and nucleic acid content of juvenile cod (*Gadus morhua* L.). Can J Zool 70: 2095-2102.

Fraser KPP, Rogers AD (2007) Protein metabolism in marine animals: the underlying mechanism of growth. Adv Mar Biol 52: 267-362.

Gabillard J-C, Weil C, Rescan P-Y, Navarro I, Gutierrez J, Le Bail P-Y (2003*a*) Effects of environmental temperature on IGF1, IGF2, and IGF type I receptor expression in rainbow trout (*Oncorhynchus mykiss*). Gen Comp Endocrinol 133: 233-242.

Gabillard J-C, Weil C, Rescan P-Y, Navarro I, Gutierrez J, Le Bail P-Y (2003*b*) Environmental temperature increases plasma GH levels independently of nutritional status in rainbow trout (*Oncorhynchus mykiss*). Gen Comp Endocrinol 133: 17-26.

Grant WS, Zhang CI, Kobayashi T, Stahl G (1987) Lack of genetic stock discretion in Pacific cod (*Gadus macrocephalus*). Can J Fish Aquat Sci 44: 490-498.

Hawkins AJS (1991) Protein turnover: a functional appraisal. Funct Ecol 5: 222-223.

Hiatt T, Dalton M, Felthoven R, Fissel B, Garber-Yonts B, Haynie A, Kasperski S, Lew D, Package C, Sepez J, Seung C (2010) Stock assessment and fishery evaluation report for the groundfish fisheries of the Gulf of Alaska and Bering Sea/Aleutian Islands area: Economic status of the groundfish fisheries off Alaska, 2009. Published at http://www.afsc.noaa.gov/refm/docs/2010/economic.pdf

Houlihan DF, Pedersen BH, Steffensen JF, Brechin J (1995) Protein synthesis, growth, and energetics in larval herring (*Clupea harengus*) at different feeding regimes. Fish Physiol Biochem14: 195-208.

Houlihan DF, Hall SJ, Gray C, Noble BS (1988) Growth rates and protein turnover in Atlantic cod, *Gadus morhua.* Can J Fish Aquat Sci 45: 951-964.

Houlihan DF, Wieser W, Foster A, Brechin J (1992) *In vitro* protein synthesis rates in larval nase (*Chondrostoma nasus* L.). Can J Zool 70: 2436-2440.

Hurst TP, Cooper DW, Scheingross JS, Seale EM, Laurel BJ, Spencer ML (2009) Effects of ontogeny, temperature, and light on vertical movements of larval Pacific cod (*Gadus macrocephalus*). Fish Oceanogr 18: 301-311.

Hurst TP, Laurel BJ, Cianelli L (2010) Ontogenetic patterns and temperature-dependent growth rates in early life stages of Pacific cod (*Gadus macrocephalus*). Fish Bull 108: 382-392.

Hunt von Herbing I, Boutilier RG, Miyake T, Hall BK (1996) Effects of temperature on morphological landmarks critical to growth and survival in larval Atlantic cod (*Gadus morhua*). Mar Biol 124: 593-606.

Janhunen M, Piironen J, Peuhkuri N (2010) Parental effects on embryonic viability and growth in Arctic charr *Salvelinus alpinus* at two incubation temperatures. J Fish Biol 76: 2558-2570.

Jewett SC (1978) Summer food of the Pacific cod, *Gadus macrocephalus*, near Kodiak Island, Alaska. Fish Bull 76: 700-706.

Jonsson N, Jonsson B, Hansen LP (2005) Does climate during embryonic development influence parr growth and age of seaward migration in Atlantic salmon (*Salmo salar*)? Can J Fish Aquat Sci 62: 2502-2508.

Kamler E (2002) Ontogeny of yolk-feeding fish: an ecological perspective. Rev Fish Biol Fish 12: 79-103.

Kamler E (2008) Resource allocation in yolk-feeding fish. Rev Fish Biol Fish 18: 143-200.

Ketchen KS (1961) Observations on the ecology of the Pacific cod (*Gadus macrocephalus*) in Canadian waters. J Fish Res Board Can 18: 513-558.

Koedijk RM, Le Francois NR, Blier PU, Foss A, Folkvord A, Ditlecadet D, Lamarre SG, Stefansson SO, Imsland AK (2010*a*) Ontogenetic effects of diet during early development on growth performance, myosin mRNA expression and metabolic enzyme activity in Atlantic cod juveniles reared at different salinities. Comp Biochem Physiol A 156: 102-109.

Koedijk RM, Folkvord A, Foss A, Pittman K, Stefansson SO, Handeland S, Imsland AK (2010*b*) The influence of first-feeding diet on the Atlantic cod *Gadus morhua* phenotype: survival, development, and long-term consequences for growth. J Fish Biol 77: 1-19.

Laurel BJ, Copeman LA, Hurst TP, Parrish CC (2010) The ecological significance of lipid/fatty acid synthesis in developing eggs and newly hatched larvae of Pacific cod (*Gadus macrocephalus*). Mar Biol 157: 1713-1724.

Laurel BJ, Hurst TP, Cianelli L (2011) An experimental examination of temperature interactions in the match-mismatch hypothesis for Pacific cod larvae. Can J Fish Aquat Sci 68: 51-61.

Laurel BJ, Hurst TP, Copeman LA, Davis MW (2008) The role of temperature on the growth and survival of early and late hatching Pacific cod larvae (*Gadus macrocephalus*). J Plankton Res 30: 1051-1060.

Lewis JM, Driedzic WR (2007) Tissue specific changes in protein synthesis associated with seasonal metabolic depression and recovery in the north temperate labrid, *Tautogolabrus adspersus*. Am J Physiol-Reg Integr Comp Physiol 293: R474-R481.

Li M, Leatherland J (2008) Temperature and ration effects on components of the IGF system and growth performance of rainbow trout (*Oncorhynchus mykiss*) during the transition from late stage embryos to early stage juveniles. Gen Comp Endocrinol 155: 668-679.

Mecklenburg CW, Mecklenburg TA, Thorsteinson LK (2002) Fishes of Alaska. American Fisheries Society, Maryland.

McLaughlin RL, Ferguson MM, Noakes DLG (1995) Concentrations of nucleic acids and protein as indices of nutritional status for recently emerged brook trout (*Salvelinus fontinalis*). Can J Fish Aquat Sci 52: 848-854.

McMillan DN, Houlihan DF (1988) The effect of refeeding on tissue protein synthesis in rainbow trout. Physiol Zool 61: 429-441.

Pavlov DA, Mikhaylenko VG, Timeyko VN, Konovalov Ye.S (1994) Reproduction and embryonic larval development of the char, *Salvelinus alpinus lepechini*, in lakes Onega and Ladoga. J Ichthyol 34: 42-56.

Peragon J, Barroso JB, Salguero LG, de la Higuera M, Lupianez JA (2001) Growth, protein-turnover rates and nucleic-acid concentrations in the white muscle of rainbow trout during development. Int J Biochem Cell Biol 33: 1227-1238.

Puvanendran V, Brown JA (1999) Foraging, growth and survival of Atlantic cod larvae reared in different prey concentrations. Aquaculture 175: 77-92.

Robinson SMC, Ware DM (1988) Ontogenetic development of growth rates in larval Pacific herring, *Clupea harengus pallasi*, measured with RNA-DNA ratios in the Strait of Georgia, British Columbia. Can J Fish Aquat Sci 45: 1422-1429.

Sakurai Y, Hattori T (1996) Reproductive behavior of Pacific cod in captivity. Fish Sci 62: 222-228.

Smith RW, Ottema C (2006) Growth, oxygen consumption, and protein and RNA synthesis rates in the yolk sac larvae of the African catfish (*Clarias gariepinus*). Comp Biochem Physiol A 143: 315-325.

Smith RW, Houlihan DF (1995) Protein synthesis and oxygen consumption in fish cells. J Comp Physiol B 165: 93-101.

Smith RW, Palmer RM, Houlihan DF (2000) RNA turnover and protein synthesis in fish cells. J Comp Physiol B 170: 135-144.

Sogard SM, Olla BL (1996) Diel patterns of behavior in juvenile walleye pollock, *Theragra chalcogramma*. Environ Biol Fish 47: 379-386.

Stepanenko MA (1995) Distribution, behavior, and abundance of Pacific cod, *Gadus macrocephalus*, in the Bering Sea. J Ichthyol 35: 18-27.

Stierhoff KL, Targett TE, Power JH (2009) Hypoxia-induced growth limitation of juvenile fishes in an estuarine nursery: assessment of small-scale temporal dynamics using RNA:DNA. Can J Fish Aquat Sci 66: 1033-1047.

Takatsu T, Nakatani T, Mutoh T, Takahashi T (1995) Feeding habits of Pacific cod larvae and juveniles in Mutsu bay, Japan. Fish Sci 61: 415-422.

Tanaka M, Kawai S, Seikai T, Burke JS (1996) Development of the digestive organ system in Japanese flounder in relation to metamorphosis and settlement. Mar Freshw Behav Physiol 28: 19-31.

Teletchea F, Fontaine P (2010) Comparison of early life-stage strategies in temperate freshwater fish species: trade-offs are directed towards first feeding of larvae in spring and early summer. J Fish Biol 77: 257-278.

Theilacker GH, Bailey KM, Canino MF, Porter SM (1996) Variations in larval walleye pollock feeding and condition: A synthesis. Fish Oceanogr 5 (Suppl 1): 112-123.

Tong XH, Liu QH, Xu SH, Li J, Xiao ZZ, Ma DY (2010) Changes in RNA, DNA, protein contents and growth of turbot *Scophthalmus maximus* larvae and juveniles. J Fish Biol 77: 512-525.

Treberg JR, Hall JR, Driedzic WR (2005) Enhanced protein synthetic capacity in Atlantic cod (*Gadus morhua*) is associated with temperature-induced compensatory growth. Am J Physiol-Reg Integr Comp Physiol 288: R205-R211.

Wagenmakers EJ, Farrell S (2004) AIC model selection using Akaike weights. Psychon Bull Rev 11: 192-196.

Weber LP, Higgins PS, Carlson RI, Janz DM (2003) Development and validation of methods for measuring multiple biochemical indices of condition in juvenile fishes. J Fish Biol 63: 37-658.

Westerman M, Holt GJ (1994) RNA:DNA ratio during the critical period and early larval growth of the red drum *Sciaenops ocellatus*. Mar Biol 121: 1-9.

Wieser W (1995) Energetics of fish larvae, the smallest vertebrates. Acta Physiol Scand 154: 279-290.

Wieser W, Forstner H, Medgyesy N, Hinterleitner S (1988) To switch or not to switch: partitioning of energy between growth and activity in larval cyprinids (Cyprinidae: Teleostei). Funct Ecol 2: 499-507.

Yin MC, Blaxter JHS (1986) Morphological changes during growth and starvation of larval cod (*Gadus morhua* L.) and flounder (*Platichthys flesus* L.). J Exp Mar Biol Ecol 104: 215-228.

Zouiten D, Khemis IB, Besbes R, Cahu C (2008) Ontogeny of the digestive tract of thick lipped grey mullet (*Chelon labrosus*) larvae reared in "mesocosms". Aquaculture 279: 166-172.

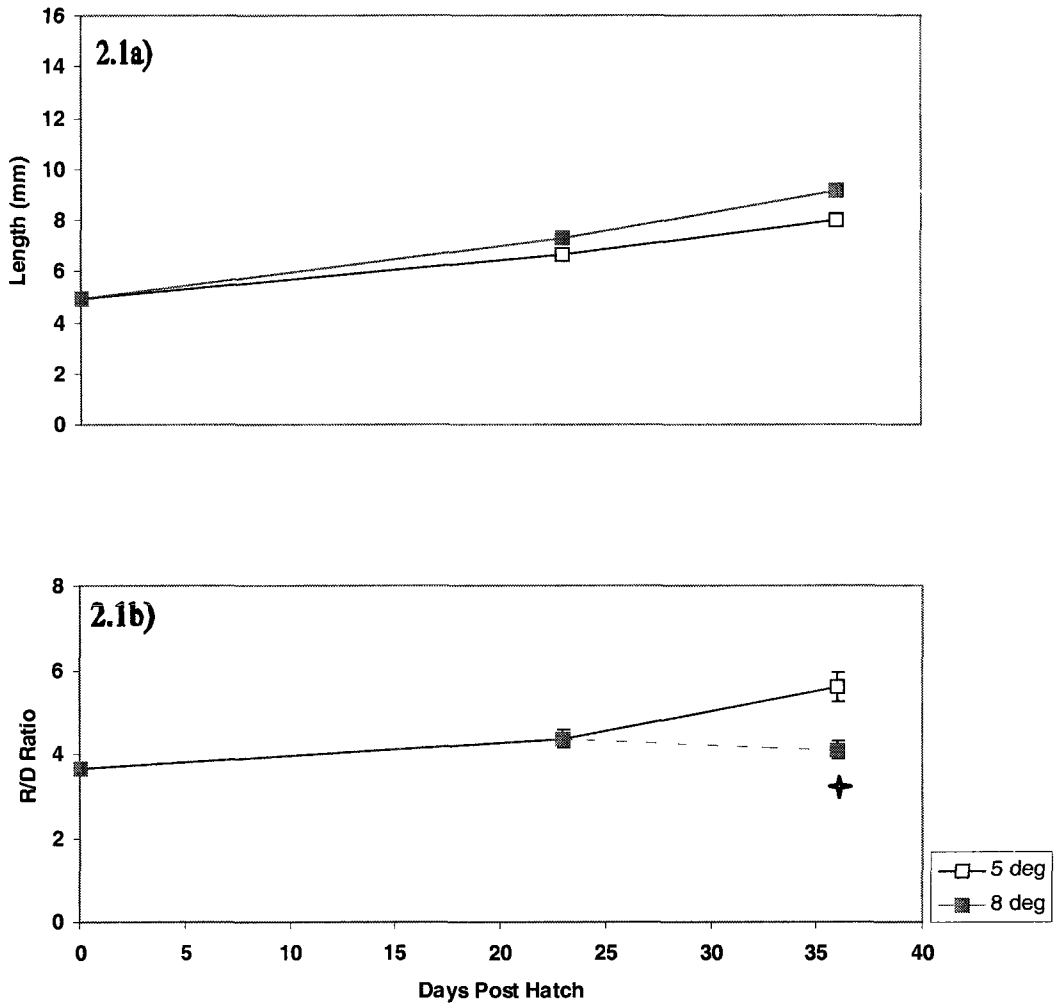

Fig. 2.1a,b: *Gadus macrocephalus*. Length and RNA/DNA in larvae at 5°C and 8°C. Comparison of length (2.1a) and RNA/DNA (R/D) ratios (2.1b) between Pacific cod larvae cultured at 5°C (open symbols) and 8°C (closed symbols). Symbol bars represent standard error. Significant differences ($p \leq 0.05$) between treatments represented by ✦

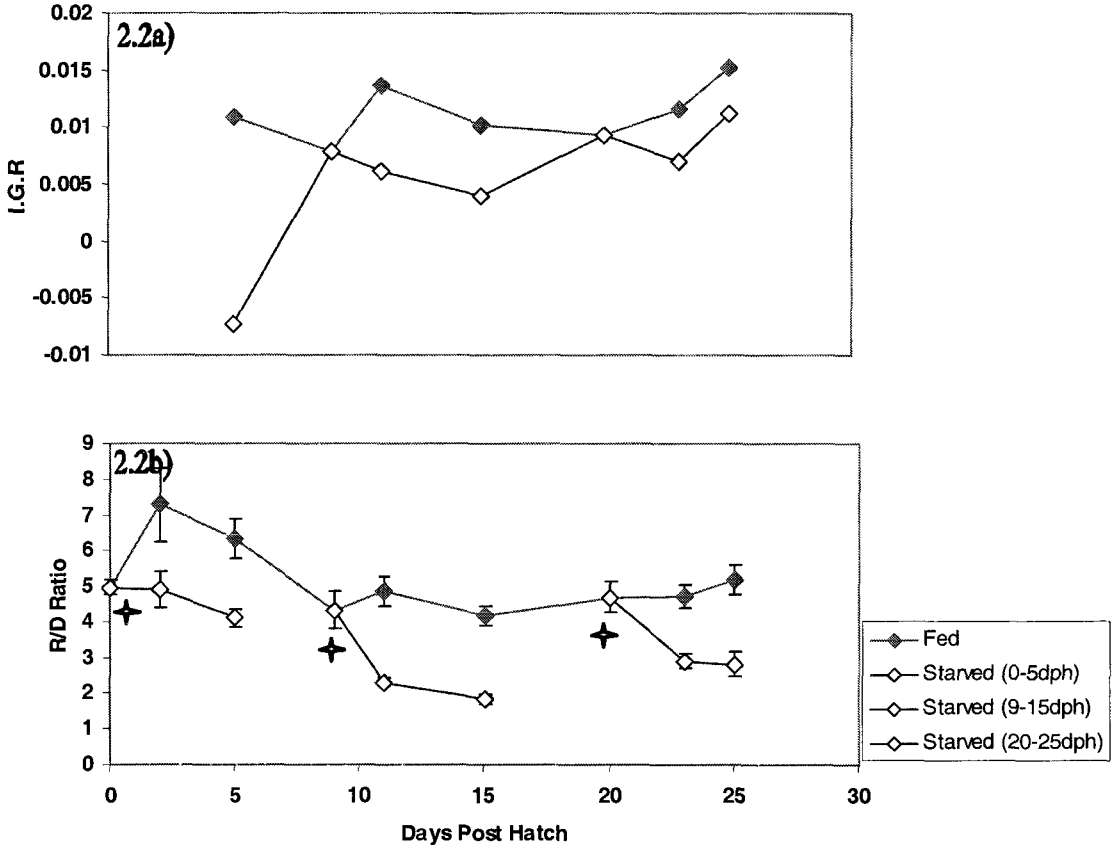

Fig. 2.2a,b: *Gadus macrocephalus*. Growth rate and RNA/DNA in fed and starved larvae. Comparison of instantaneous growth rates (I.G.R) (2.2a) and RNA/DNA (R/D) ratios (2.2b) between fed (closed symbols) and starved (open symbols) Pacific cod larvae. Symbol bars represent standard error. Significant differences ($p \leq 0.05$) between fed and fasted treatments represented by ✦

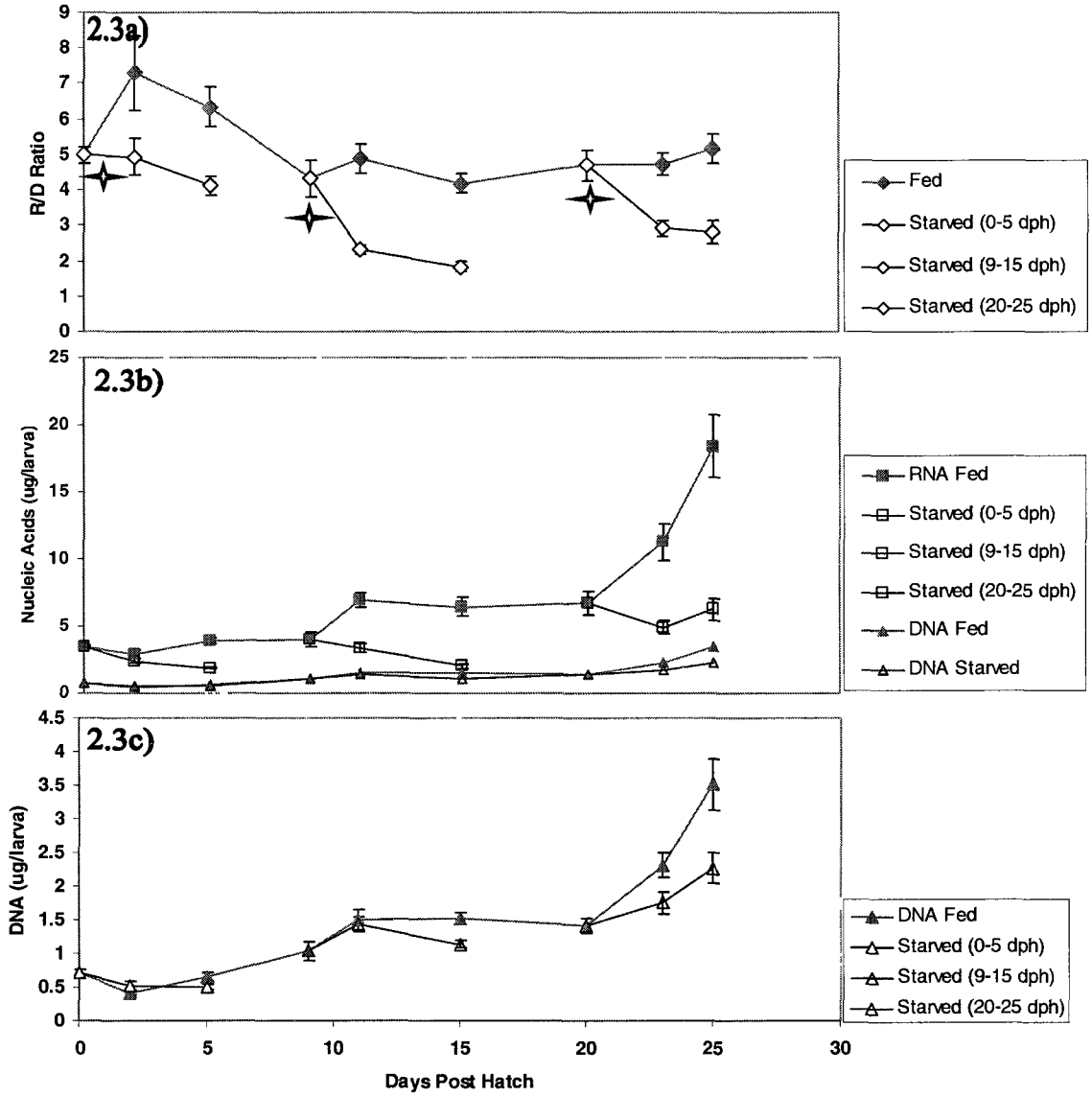

Fig. 2.3a,b,c: *Gadus macrocephalus*. Nucleic acid trends in fed and starved larvae. Comparison of RNA/DNA (R/D) ratios (2.3a), nucleic acids (RNA and DNA) (2.3b), and DNA (2.3c) between fed (closed symbols) and starved (open symbols) Pacific cod larvae from 0-25 days post hatch. Significant differences ($p \leq 0.05$) between fed and starved groups shown by ✦

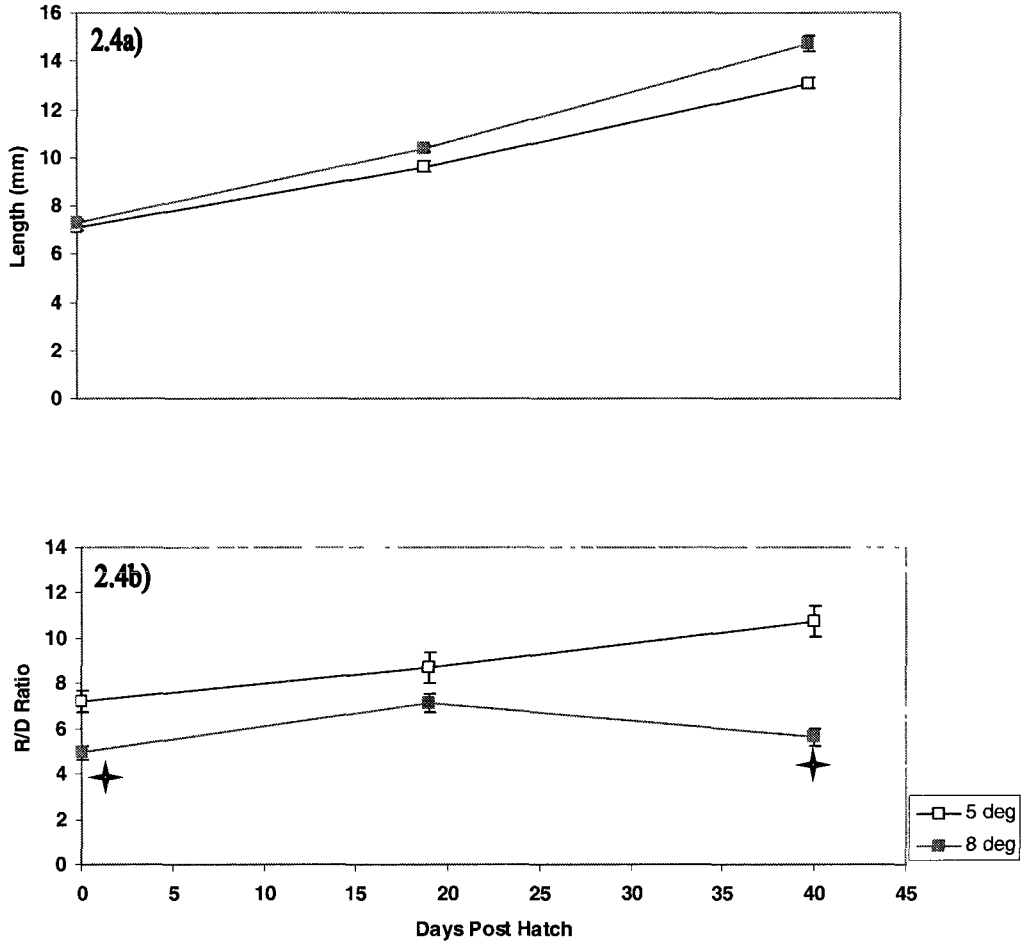

Fig. 2.4a,b: *Theragra chalcogramma*. Length and RNA/DNA in larvae at 5°C and 8°C. Comparison of length (2.4a) and RNA/DNA (R/D) ratios (2.4b) between walleye pollock larvae cultured at 5°C (open symbols) and 8°C (closed symbols). Symbol bars represent standard error. Significant differences ($p \leq 0.05$) between treatments represented by ✦

Table 2.1: *Gadus macrocephalus*. Growth in larvae at 5°C and 8°C. Statistical comparison of total length, instantaneous growth rate (I.G.R.), and RNA/DNA between Pacific cod larvae cultured at two temperature treatments (5°C, 8°C) over 0-36 days post hatch (dph). *P*-values ≤ 0.05 are statistically significant.

	Comparison over 0-36 dph	Tukey posthoc test comparison between 5°C and 8°C treatments		
		0 dph	23 dph	36 dph
Length	< 0.001	1.0000	< 0.001	< 0.001
I.G.R.	< 0.001			
RNA/DNA	0.001	0.9949	1.0000	< 0.001

Table 2.2: *Gadus macrocephalus*. Growth in fed and starved larvae at 6.5°C. Statistical comparison of instantaneous growth rate (I.G.R.), and RNA/DNA (R/D) between larval Pacific cod cultured at two nutritional states (fed and starved) at 6.5°C over 0-25 days post hatch (dph). *P*-values ≤ 0.05 are statistically significant.

Between treatment comparison over 0-25 dph		Tukey posthoc test comparison (between treatment comparison for each sampling day)								
		0 dph	2 dph	5 dph	9 dph	11 dph	15 dph	20 dph	23 dph	25 dph
I.G.R.	< 0.001									
R/D	< 0.001	1.000	0.2191	0.0061	1.0000	0.0101	0.0003	1.0000	0.0180	0.0458

Table 2.3: *Gadus macrocephalus*. Growth model regression. Multiple linear regression coefficients describing the relationship between instantaneous growth rates (I.G.R.), log RNA-$\mathbf{a_1}$, log DNA-$\mathbf{b_1}$, and temperature-$\mathbf{c_1}$ for Pacific cod larvae. Equation is of the form I.G.R. = a_1+b_1+ c_1+C, where C is a constant. The regression was significant ($p < 0.001$). SE is standard error.

Coefficients (+/- SE)	a_1	b_1	c_1	C	Adjusted r^2
Pacific cod	0.002064 (0.002950)	0.003922 (0.003243)	0.0002160 (0.0004581)	0.004515 (0.006022)	0.655

Table 2.4: *Gadus macrocephalus*. AIC_C comparison. Model selection by second-order Akaike Information Criterion (AIC_C) values. Coefficients (± SE) in alternative regression models of growth in larval Pacific cod. In all models, $p < 0.001$. Log R/D = Log RNA/DNA ratio, T = treatment temperature, K = number of model parameters (including intercept), Δ AIC = difference in AIC with respect to the best fit model.

	Model Parameters						Intercept	Adjusted r^2	K	AIC_C	Δ AIC
#	RNA	Log RNA	DNA	Log DNA	Log R/D	T					
1		0.0020 (0.0029)		0.0039 (0.0032)		0.0002 (0.0004)	0.004515	0.655	4	-122	0
2	0.0002 (0.0002)		0.0012 (0.0013)			0.0002 (0.0005)	0.006610	0.539	4	-119	3
3					0.0035 (0.0049)	0.0014 (0.0006)	-0.00175	0.127	3	-114	8

Table 2.5: *Theragra chalcogramma*. Growth in larvae at 5°C and 8°C. Statistical comparison of total length, instantaneous growth rate, and RNA/DNA between walleye pollock larvae cultured at two temperature treatments (5°C, 8°C) over 0-40 days post hatch (dph). *P*-values ≤ 0.05 are statistically significant.

	Comparison over 0-40 dph	Tukey posthoc test comparison between 5°C and 8°C treatments		
		0 dph	19 dph	40 dph
Length	< 0.001	0.9447	0.0899	< 0.001
I.G.R.	< 0.001			
R/D	< 0.001	0.0050	0.2333	< 0.001

General Conclusion

These studies highlight the potential utility and applications of nucleic acids in observing and interpreting cellular growth responses in fish. Growth responses in Pacific cod, walleye pollock, and Pacific herring are affected by variations in temperature and diet. Physiological growth assessments could be relevant in understanding fish growth and condition in response to variations in temperature and diet, and R/D ratios have potential as a growth index in determining physiological growth assessments. This index was sensitive enough to observe growth responses and physiological strategies in larval and juvenile fish, and could enable energetic assessments that have possible applications in predictive population and growth models. This could be especially relevant to high-value fish species in Alaskan waters, such as Pacific cod, walleye pollock, and Pacific herring.

While R/D ratios and nucleic acid-based growth models can have certain limitations in their applications, as described earlier, the advantages are numerous. Nucleic acid ratios are applicable as a growth index across different fish species, age classes, and tissue types. Most importantly, the results of these studies suggest that R/D should not be viewed as a substitute for other growth indices, but instead can complement other physiological growth indices, such as lipid and protein concentrations. When possible, R/D should be integrated with other growth indices to give a comprehensive view of seasonal fish growth. Growth indices like R/D may help in observing initial cellular responses to environmental variation, and in concert with lipid and protein concentrations, could potentially allow an understanding of how fish growth and

condition may be affected by changes in temperature and diet. Growth models based on nucleic acids may have applications in predicting growth rates of individual fish without prior condition measures. These growth models could have management applications, since growth and condition estimates are required for recruitment and stock assessments.

Using R/D in concert with other growth indices could be very useful in studying the growth of species in the North Pacific Ocean. Specifically with regard to YOY Pacific herring, the use of R/D ratios highlighted compensation in steps that precede and could be essential to CG. Nucleic acid ratios also have potential as an index in terms of growth/mortality thresholds, and in conjunction with soluble protein and total body lipid, suggested a photoperiod-influenced seasonal change of resource allocation and partitioning in Pacific herring. The use of R/D in larval Pacific cod and walleye pollock showed that growth responses in these fish could be affected by small differences in temperature, with possible compensatory growth strategies. Changes in R/D, RNA, and DNA could also be used to identify distinct stanzas in early growth of Pacific cod larvae. While it might not be feasible, or even required, to conduct similar studies on numerous fish species in the North Pacific Ocean, the focus of research can be narrowed to species of interest, i.e., those of high ecological and commercial value. Utilizing R/D in concert with growth indices already widely used could increase our understanding of seasonal growth and condition in these chosen species.

Literature Cited

Abookire AA, Piatt JF, Norcross BL (2001) Juvenile groundfish habitat in Kachemak Bay, Alaska, during late summer. Alsk Fish Res Bull 8: 45-56.

Adams MS (1999) Ecological role of lipids in the health and success of fish populations. In: Arts TM, Wainman BC (eds) Lipids in Freshwater Ecosystems. Springer-Verlag, New York: 132-160.

Albers WD, Anderson PJ (1985) Diet of Pacific cod, *Gadus macrocephalus*, and predation on the northern pink shrimp, *Pandalus borealis*, in Pavlof Bay, Alaska. Fish Bull 83: 601-610.

Alderdice DF, Forrester CR (1971) Effects of salinity, temperature, and dissolved oxygen on early development of the Pacific cod (*Gadus macrocephalus*). J Fish Res Board Can 28: 883-902.

Alderdice DF, Velsen FPJ (1971) Some effects of salinity and temperature on early development of Pacific herring (*Clupea pallasi*). J Fish Res Board Can 28: 1545-1562.

Anderson PJ, Blackburn JE, Johnson BA (1997) Declines of forage species in the Gulf of Alaska, 1972-95, as indicator of regime shift. *In*: Baxter B.S. (ed). Proceedings of the International Symposium on the Role of Forage Fishes in Marine Ecosystems November 13-16, 1996, Anchorage, Alaska. University of Alaska Sea Grant Rep 97-01: 531-543.

Anderson PJ, Piatt JF (1999) Community reorganization in the Gulf of Alaska following ocean climate regime shift. Mar Ecol Prog Ser 189: 117-123.

Angilletta Jr. MJ, Wilson RS, Navas CA, James RS (2003) Tradeoffs and the evolution of thermal reaction norms. Trends Ecol Evol 18: 234-240.

Atkinson D (1994) Temperature and organism size-a biological law for ectotherms? Adv Ecol Res 25: 1-58.

Bacheler NM, Ciannelli L, Bailey KM, Duffy-Anderson JT (2010) Spatial and temporal patterns of walleye pollock (*Theragra chalcogramma*) spawning in the eastern Bering Sea inferred from egg and larval distributions. Fish Oceanogr 19: 107-120.

Bailey KM, Stabeno PJ, Powers DA (1997) The role of larval retention and transport features in mortality and potential gene flow of walleye pollock. J Fish Biol 51 (Suppl. A): 135-154.

Bakkala RG (1984) Pacific cod of the eastern Bering Sea. Bull Int North Pac Fish Comm 42: 157-179.

Bakkala RG, Mishima S, Westrheim SJ, Zhang CI, Brown ES (1984) Distribution of Pacific cod (*Gadus macrocephalus*) in the North Pacific Ocean. Bull Int North Pac Fish Comm 42: 111-115.

Bauchat JR, Busby WH Jr., Garmong A, Swanson P, Moore J, Lin M, Duan C (2001) Biochemical and functional analysis of a conserved IGF-binding protein isolated from rainbow trout (*Oncorhynchus mykiss*) hepatoma cells. J Endocrinol 170: 619-628.

Beamish RJ, Benson AJ, Sweeting RM, Neville CM (2004) Regimes and the history of the major fisheries off Canada's west coast. Prog Oceanogr 60: 355-385.

Beamish RJ, Bouillon DR (1993) Pacific salmon production trends in relation to climate. Can J Fish Aquat Sci 50: 1002-1016.

Benson AJ, Trites AW (2002) Ecological effects of regime shifts in the Bering Sea and eastern North Pacific Ocean. Fish and Fisheries 3: 95-113.

Boutilier RG (1998) Physiological ecology in cold ocean fisheries: a case study in Atlantic cod. In *Cold Ocean Physiology* (eds. Portner, H.O., and Playle, R.), pp. 463-489. Cambridge University Press, Cambridge.

Bradshaw WE, Holzapfel CM (2010) Light, time and the physiology of biotic response to rapid climatic change in animals. Annu Rev Physiol 72: 147-166

Brodeur RD, Ware DM (1992) Long-term variability in zooplankton biomass in the subArctic Pacific Ocean. Fish Oceanogr 1: 32-38.

Brodeur RD, Wilson MT, Ciannelli L (2000) Spatial and temporal variability in feeding and condition of age-0 walleye pollock (*Theragra chalcogramma*) in frontal regions of the Bering Sea. ICES J Mar Sci 57: 256-264.

Buckley LJ (1979) Relationships between RNA-DNA ratio, prey density, and growth rate in Atlantic cod (*Gadus morhua*) larvae. J Fish Res Board Can 36: 1497-1502.

Buckley LJ (1984) RNA-DNA ratio: an index of larval fish growth in the sea. Mar Biol 80: 291-298.

Buckley LJ, Caldarone E, Ong TL (1999) RNA-DNA ratio and other nucleic acid-based indicators for growth and condition of marine fishes. Hydrobiologia 401: 265-277.

Busacker GP, Adelman IR, Goolish EM (1990) Growth. In *Methods for Fish Biology* (eds. Schreck CB and Moyle PB), pp 363-388. American Fisheries Society, Bethesda, Maryland.

Caldarone EM (2005) Estimating growth in haddock larvae *Melanogrammus aeglefinus* from RNA:DNA ratios and water temperature. Mar Ecol Prog Ser 293: 241-252

Caldarone, EM, St. Onge-Burns JM, Buckley LJ (2003) Relationship of RNA/DNA ratio and temperature to growth in larvae of Atlantic cod *Gadus morhua*. Mar Ecol Prog Ser 262: 229-240.

Carlson HR (1980) Seasonal distribution and environment of Pacific herring near Auke Bay, Lynn Canal, southeastern Alaska. Trans Am Fish Soc 109: 71-78.

Cheung WWL, Lam VWY, Sarmiento JL, Kearney K, Watson R, Pauly D (2009) Projecting global marine biodiversity impacts under climate change scenarios. Fish and Fisheries 10: 235-251.

Ciannelli L, Bailey KM, Chan K-S, Stenseth NC (2007) Phenological and geographical patterns of walleye pollock (*Theragra chalcogramma*) spawning in the western Gulf of Alaska. Can J Fish Aquat Sci 64: 713-722.

Ciannelli L, Brodeur RD, Napp JM (2004) Foraging impact on zooplankton by age-0 walleye pollock (*Theragra chalcogramma*) around a front in the southeast Bering Sea. Mar Biol 144: 515-526.

Claireaux G, Webber DM, Kerr SR, Boutilier RG (1995*a*) Physiology and behaviour of free-swimming Atlantic cod (*Gadus morhua*) facing fluctuating temperature conditions. J Exp Biol 198: 49-60.

Claireaux G, Webber DM, Kerr SR, Boutilier RG (1995*b*) Physiology and behaviour of free-swimming Atlantic cod (*Gadus morhua*) facing fluctuating salinity and oxygenation conditions. J Exp Biol 198: 61-69.

Claireaux G, Webber DM, Lagardere JP, Kerr SR (2000) Influence of water temperature and oxygenation on the aerobic metabolic scope of Atlantic cod (*Gadus morhua*). J Sea Res 44: 257-265.

Colton AR, Hurst TP (2010) Behavioral responses to light gradients, olfactory cues, and prey in larvae of two North Pacific gadids (*Gadus macrocephalus* and *Theragra chalcogramma*). Environ Biol Fish 88: 39-49.

Couture P, Dutil JD, Guderley H (1998) Biochemical correlates of growth and condition in juvenile Atlantic cod (*Gadus morhua*) from Newfoundland. Can J Fish Aquat Sci 55: 1591-1598.

Dahlhoff E, Somero GN (1993) Effects of temperature on mitochondria from abalone (genus *Haliotis*): adaptive plasticity and its limits. J Exp Biol 185: 151-168.

Daufresne M, Lengfellner K, Sommer U (2009) Global warming benefits the small in aquatic ecosystems. PNAS 106: 12788-12793.

DeBlois EM, Rose GA (1995) Effect of foraging activity on the shoal structure of cod (*Gadus morhua*). Can J Fish Aquat Sci 52: 2377-2387.

Duan C (1998) Nutritional and developmental regulation of insulin-like growth factors in fish. J Nutr 128: 306S-314S.

Duan C, Ding J, Li Q, Tsai W, Pozios K (1999) Insulin-like growth factor binding protein 2 is a growth inhibitory protein conserved in zebrafish. PNAS 96: 15274-15279.

Engelhard GH, Heino M (2005) Scale analysis suggests frequent skipping of the second reproductive season in Atlantic herring. Biol Lett 1: 172-175.

Engelhard GH, Heino M (2006) Climate change and condition of herring (*Clupea harengus*) explain long-term trends in extent of skipped reproduction. Oecologia 149: 593-603.

Farrell AP (2009) Environment, antecedents and climate change: lessons from the study of temperature physiology and river migration of salmonids. J Exp Biol 212: 3771-3780.

Flath LE, Diana JS (1985) Seasonal energy dynamics of the alewife in southeastern Lake Michigan. Trans Am Fish Soc 114: 328-337.

Forister ML, McCall AC, Sanders NJ, Fordyce JA, Thorne JH, O'Brien J, Waetjen DP, Shapiro AM (2010) Compounded effects of climate change and habitat alteration shift patterns of butterfly diversity. PNAS 107: 2088-2092.

Foy RJ, Norcross BL (1999) Spatial and temporal variability in the diet of juvenile Pacific herring (*Clupea pallasi*) in Prince William Sound, Alaska. Can J Zool 77: 697-706.

Foy RJ, Paul AJ (1999) Winter feeding and changes in somatic energy content of age-0 Pacific herring in Prince William Sound, Alaska. Trans Am Fish Soc 128: 1193-1200.

Francis RC, Hare SR (1994) Decadal-scale regime shifts in the large marine ecosystems of the Northeast Pacific: a case for historical science. Fish Oceanogr 3: 279-291.

Francis RC, Hare SR, Hollowed AB, Wooster WS (1998) Effects of interdecadal climate variability on the oceanic ecosystems of the NE Pacific. Fish Oceanogr 7: 1-21.

Fraser KPP, Rogers AD (2007) Protein metabolism in marine animals: the underlying mechanism of growth. Adv Mar Biol 52: 267-362.

Freeland HJ (1990) Sea surface temperatures along the coast of British Columbia: regional evidence for a warming trend. Can J Fish Aquat Sci 47: 346-350.

Fudge DS, Ballantyne JS, Stevens ED (2001) A test of biochemical symmorphosis in a heterothermic tissue: bluefin tuna white muscle. Am J Physiol 280: R108-R114.

Fudge DS, Stevens ED, Ballantyne JS (1997) Enzyme adaptation along a heterothermic tissue: the visceral retia mirabilia of the bluefin tuna. Am J Physiol 272: R1834-R1840.

Gabillard J-C, Weil C, Rescan P-Y, Navarro I, Gutierrez J, Le Bail P-Y (2003*a*) Effects of environmental temperature on IGF1, IGF2, and IGF type I receptor expression in rainbow trout (*Oncorhynchus mykiss*). Gen Comp Endocrinol 133: 233-242.

Gabillard J-C, Weil C, Rescan P-Y, Navarro I, Gutierrez J, Le Bail P-Y (2003*b*) Environmental temperature increases plasma GH levels independently of nutritional status in rainbow trout (*Oncorhynchus mykiss*). Gen Comp Endocrinol 133: 17-26.

Gabillard J-C, Kamangar BB, Montserrat N (2006) Coordinated regulation of the GH/IGF system genes during refeeding in rainbow trout (*Oncorhynchus mykiss*). J Endocrinol 191: 15-24.

Grant WS, Zhang CI, Kobayashi T, Stahl G (1987) Lack of genetic stock discretion in Pacific cod (*Gadus macrocephalus*). Can J Fish Aquat Sci 44: 490-498.

Guderley H (2004) Metabolic responses to low temperature in fish muscle. Biol Rev 79: 409-427.

Guderley H, Dutil JD, Pelletier D (1996) The physiological status of Atlantic cod, *Gadus morhua*, in the wild and the laboratory: estimates of growth rates under field conditions. Can J Fish Aquat Sci 53: 550-557.

Guderley H, Lapointe D, Bedard M, Dutil JD (2003) Metabolic priorities during starvation: enzyme sparing in liver and white muscle of Atlantic cod, *Gadus morhua* L. Comp Biochem Physiol A 135: 347-356.

Haegele CW, Schweigert JF (1985) Distribution and characteristics of herring spawning grounds and description of spawning behavior. Can J Fish Aquat Sci 42 (Suppl 1): 39-55.

Hay DE (1985) Reproductive biology of Pacific herring (*Clupea harengus pallasi*). Can J Fish Aquat Sci 42 (Suppl. 1): 111-126.

Hazel JR (1995) Thermal adaptation in biological membranes: Is homeoviscous adaptation the explanation? Annu Rev Physiol 57: 19-42.

Hiatt T, Dalton M, Felthoven R, Fissel B, Garber-Yonts B, Haynie A, Kasperski S, Lew D, Package C, Sepez J, Seung C (2010) Stock assessment and fishery evaluation report for the groundfish fisheries of the Gulf of Alaska and Bering Sea/Aleutian Islands area: Economic status of the groundfish fisheries off Alaska, 2009. Published at http://www.afsc.noaa.gov/refm/docs/2010/economic.pdf

Hiddink, JG, Ter Hofstede R (2008) Climate induced increases in species richness of marine fishes. Global Change Biol 14: 453-460.

Hinckley S (1987) The reproductive biology of walleye pollock, *Theragra chalcogramma*, in the Bering Sea, with reference to spawning stock structure. Fish Bull 85: 481-498.

Hoegh-Guldberg O, Bruno JF (2010) The impact of climate change on the world's marine ecosystems. Science 328: 1523-1528.

Hofmann GE, Todgham AE (2010) Living in the now: physiological mechanisms to tolerate a rapidly changing environment. Annu Rev Physiol 72: 127-145.

Hollowed AB, Wooster WW (1992) Variability of winter ocean conditions and strong year classes of northeast Pacific groundfish. ICES Mar Sci Symp 195: 433-444.

Houlihan DF, Pedersen BH, Steffensen JF, Brechin J (1995) Protein synthesis, growth, and energetics in larval herring (*Clupea harengus*) at different feeding regimes. Fish Physiol Biochem14: 195-208.

Houde ED (1989) Comparative growth, mortality, and energetics of marine fish larvae: temperature and implied latitudinal effects. Fish Bull 87: 471-495.

Hurst TP, Cooper DW, Scheingross JS, Seale EM, Laurel BJ, Spencer ML (2009) Effects of ontogeny, temperature, and light on vertical movements of larval Pacific cod (*Gadus macrocephalus*). Fish Oceanogr 18: 301-311.

Hurst TP, Laurel BJ, Cianelli L (2010) Ontogenetic patterns and temperature-dependent growth rates in early life stages of Pacific cod (*Gadus macrocephalus*). Fish Bull 108: 382-392.

Jewett SC (1978) Summer food of the Pacific cod, *Gadus macrocephalus*, near Kodiak Island, Alaska. Fish Bull 76: 700-706.

Jonsson N, Jonsson B, Hansen LP (2005) Does climate during embryonic development influence parr growth and age of seaward migration in Atlantic salmon (*Salmo salar*)? Can J Fish Aquat Sci 62: 2502-2508.

Ketchen KS (1961) Observations on the ecology of the Pacific cod (*Gadus macrocephalus*) in Canadian waters. J Fish Res Board Can 18: 513-558.

Klovach NV, Rovnina OA, Kol'tsov DV (1995) Biology and exploitation of Pacific Cod, *Gadus macrocephalus*, in the Anadyr-Navarin region of the Bering Sea. J Ichthyol 35: 48-52.

Lang GM, Brodeur RD, Napp JM, Schabetsberger R (2000) Variation in groundfish predation on juvenile walleye pollock relative to hydrographic structure near the Pribilof Islands, Alaska. ICES J Mar Sci 57: 265-271.

Laurel BJ, Copeman LA, Hurst TP, Parrish CC (2010) The ecological significance of lipid/fatty acid synthesis in developing eggs and newly hatched larvae of Pacific cod (*Gadus macrocephalus*). Mar Biol 157: 1713-1724.

Laurel BJ, Hurst TP, Cianelli L (2011) An experimental examination of temperature interactions in the match-mismatch hypothesis for Pacific cod larvae. Can J Fish Aquat Sci 68: 51-61.

Laurel BJ, Hurst TP, Copeman LA, Davis MW (2008) The role of temperature on the growth and survival of early and late hatching Pacific cod larvae (*Gadus macrocephalus*). J Plankton Res 30: 1051-1060.

Lewis JM, Driedzic WR (2007) Tissue specific changes in protein synthesis associated with seasonal metabolic depression and recovery in the north temperate labrid, *Tautogolabrus adspersus*. Am J Physiol-Reg Integr Comp Physiol 293: R474-R481.

Li M, Leatherland J (2008) Temperature and ration effects on components of the IGF system and growth performance of rainbow trout (*Oncorhynchus mykiss*) during the transition from late stage embryos to early stage juveniles. Gen Comp Endocrinol 155: 668-679.

Livingston PA (1993) Importance of predation by groundfish, marine mammals and birds on walleye pollock *Theragra chalcogramma* and Pacific herring *Clupea pallasi* in the eastern Bering Sea. Mar Ecol Prog Ser 102: 205-215.

MacLean SA, Caldarone EM (2008) Estimating recent growth rates of Atlantic salmon smolts using RNA–DNA ratios from nonlethally sampled tissues. Trans Am Fish Soc 137: 1279-1284.

Mantua NJ, Hare SR (2002) The Pacific Decadal Oscillation. J Oceanogr 58: 35-44.

Mantua NJ, Hare SR, Zhang Y, Wallace JM, Francis RC (1997) A Pacific interdecadal climate oscillation with impacts on salmon production. Bull Am Meteorol Soc 78: 1069-1079

Mantzouni I, MacKenzie BR (2010) Productivity responses of a widespread marine piscivore, *Gadus morhua*, to oceanic thermal extremes and trends. Proc R Soc Lond B 277: 1867-1874.

Martinez M, Guderly H, Nelson JA, Webber D, Dutil JD (2002) Once a fast cod, always a fast cod: maintenance of performance hierarchies despite changing food availability in cod (*Gadus morhua*). Physiol Biochem Zool 75: 90-100.

Martinez M, Guderley H, Dutil JD, Winger PD, He P, Walsh SJ (2003) Condition, prolonged swimming performance and muscle metabolic capacities of cod *Gadus morhua*. J Exp Biol 206: 503-511.

Marty GD, Hulson P-J F, Miller SE, Quinn II TJ, Moffitt SD, Merizon RA (2010) Failure of population recovery in relation to disease in Pacific herring. Dis Aquat Org 90: 1-14.

McFarlane GA, King JR, Beamish RJ (2000) Have there been recent changes in climate? Ask the fish. Prog Oceanogr 47: 147-169.

McGurk MD, Paul AJ, Coyle KO, Ziemann DA, Haldorson LJ (1993) Relationships between prey concentrations and growth, condition, and mortality of Pacific herring, *Clupea pallasi*, larvae in an Alaskan subarctic embayment. Can J Fish Aquat Sci 50: 163-180.

McLaughlin RL, Ferguson MM, Noakes DLG (1995) Concentrations of nucleic acids and protein as indices of nutritional status for recently emerged brook trout (*Salvelinus fontinalis*). Can J Fish Aquat Sci 52: 848-854.

Mecklenburg CW, Mecklenburg TA, Thorsteinson LK (2002) Fishes of Alaska. American Fisheries Society, Maryland.

Mieszkowska N, Genner MJ, Hawkins SJ, Sims DW (2009) Effects of climate change and commercial fishing on Atlantic cod *Gadus morhua*. Adv Mar Biol 56: 213-273.

Minobe S (1997) A 50-70 year climatic oscillation over the North Pacific and North America. Geophys Res Lett 24: 683-686.

Mueter FJ, Litzow MA (2008) Sea ice retreat alters the biogeography of the Bering Sea continental shelf. Ecol Appl 18: 309-320.

Mueter FJ, Norcross BL (1999) Linking community structure of small demersal fishes around Kodiak Island, Alaska, to environmental variables. Mar Ecol Prog Ser 190: 37-51

Napp JM, Kendall Jr. AW, Schumacher JD (2000) A synthesis of biological and physical processes affecting the feeding environment of larval walleye pollock (*Theragra chalcogramma*) in the eastern Bering Sea. Fish Oceanogr 9: 147-162.

Norcross BL, Hose JE, Frandsen M, Brown ED (1996) Distribution, abundance, morphological condition, and cytogenic abnormalities of larval herring in Prince William Sound, Alaska, following the *Exxon Valdez* oil spill. Can J Fish Aquat Sci 53: 2376-2387.

Norcross BL, Brown ED, Foy RJ, Gay SM, Kline Jr. TC, Mason DM, Patrick EV, Paul AJ, Stokesbury KDE (2001) A synthesis of the life history and ecology of juvenile Pacific herring in Prince William Sound, Alaska. Fish Oceanogr 10 (Suppl. 1): 42-57.

Overland JE, Adams JM, Bond NA (1999) Decadal variability of the Aleutian low and its relation to high latitude circulation. J Clim 12: 1542-1548.

Overland JE, Stabeno PJ (2004) Is the climate of the Bering Sea warming and affecting the ecosystem? EOS Transactions, American Geophysical Union 85 (33): 309-316.

Parmesan C, Yohe H (2003) A globally coherent fingerprint of climate change impacts across natural systems. Nature 421: 37-42.

Paul AJ, Paul JM (1999) Interannual and regional variations in body length, weight, and energy content of age-0 Pacific herring from Prince William Sound, Alaska. J Fish Biol 54: 996-1001.

Paul AJ, Paul JM, Smith JL (1990) Consumption, growth and evacuation in the Pacific cod, *Gadus macrocephalus*. J Fish Biol 37: 117-124.

Pelletier D, Dutil JD, Blier P, Guderley H (1994) Relation between growth rate and metabolic organization of white muscle, liver, and digestive tract in cod, *Gadus morhua*. Journal of Comparative Physiology B 164: 179-190.

Pelletier D, Guderley H, Dutil JD (1993*a*) Effects of growth rate, temperature, season, and body size on glycolytic enzyme activities in the white muscle of Atlantic cod (*Gadus morhua*). The Journal of Experimental Zoology 265: 477-487.

Pelletier D, Guderley H, Dutil JD (1993*b*) Does the aerobic capacity of fish muscle change with growth rates? Fish Physiol Biochem 12: 83-93.

Pelletier D, Blier PU, Dutil JD, Guderley H (1995) How should enzyme activities be used in fish growth studies? J Exp Biol 198: 1493-1497.

Pepin P, Orr DC, Anderson JT (1997) Time to hatch and larval size in relation to temperature and egg size in Atlantic cod (*Gadus morhua*). Can J Fish Aquat Sci 54 (Suppl 1): 2-10.

Perry AL, Low PJ, Ellis JR, Reynolds JD (2005) Climate change and distribution shifts in marine fishes. Science 308: 1912-1915.

Picha EM, Turano MJ, Beckman BR, Borski RJ (2008) Endocrine Biomarkers of growth and applications to aquaculture: A minireview of growth hormone, insulin-like growth factor (IGF)-I and IGF-binding proteins as potential growth indicators in fish. North Am J Aquac 70: 196-211.

Portner HO, Bennett AF, Bozinovic F, Clarke A, Lardies MA, Lucassen M, Pelster B, Schiemer F, Stillman JH (2006) Trade-offs in thermal adaptation: the need for a molecular to ecological integration. Physiol Biochem Zool 79: 295-313.

Portner HO, Knust R (2007) Climate change affects marine fishes through the oxygen limitation of thermal tolerance. Science 315: 95-97.

Portner HO, Peck L, Somero G (2007) Thermal limits and adaptation in marine Antarctic ectotherms: an integrative view. Philos Trans R Soc Lond B 362: 2233-2258.

Post JR, Evans DO (1989) Size-dependent overwinter mortality of young-of-the-year yellow perch (*Perca flavescens*): laboratory, in situ enclosure, and field experiments. Can J Fish Aquat Sci 46: 1958-1968.

Robinson SMC, Ware DM (1988) Ontogenetic development of growth rates in larval Pacific herring, *Clupea harengus pallasi*, measured with RNA-DNA ratios in the Strait of Georgia, British Columbia. Can J Fish Aquat Sci 45: 1422-1429.

Root TL, Price JT, Hall KR, Schneider SH, Rosenzweig C, Pounds A (2003) Fingerprints of global warming on wild animals and plants. Nature 421: 57-60.

Rovnina OA, Klovach NV, Glubokov AI, Selyutin AP (1997) On the biology of Pacific cod *Gadus macrocephalus*, in the eastern part of the Sea of Okhotsk. J Ichthyol 37: 21-26.

Sakurai Y, Hattori T (1996) Reproductive behavior of Pacific cod in captivity. Fish Sci 62: 222-228.

Shimada AM, Kimura DK (1994) Seasonal movements of Pacific cod, *Gadus macrocephalus*, in the eastern Bering Sea and adjacent waters based on tag-recapture data. Fish Bull 92: 800-816.

Shimizu M, Dickey JT, Fukada H, Dickhoff WW (2005) Salmon serum 22 kDa insulin-like growth factor-binding protein (IGFBP) is IGFBP-1. J Endocrinol 184: 267-276.

Shimizu M, Swanson P, Hara A, Dickhoff WW (2003) Purification of a 41-kDa insulin-like growth factor binding protein from serum of chinook salmon, *Oncorhynchus tshawytscha*. Gen Comp Endocrinol 132: 103-111.

Sinclair EH, Vlietstra LS, Johnson DS, Zeppelin TK, Byrd GV, Springer AM, Ream RR, Hunt Jr. GL (2008) Patterns in prey use among fur seals and seabirds in the Pribilof Islands. Deep-Sea Res Part II 55: 1897-1918.

Smith TR, Buckley LJ (2003) RNA–DNA ratio in scales from juvenile cod provides a nonlethal measure of feeding condition. Trans Am Fish Soc 132: 9-17.

Sogard SM, Olla BL (1996) Diel patterns of behavior in juvenile walleye pollock, *Theragra chalcogramma*. Environ Biol Fish 47: 379-386.

Stepanenko MA (1995) Distribution, behavior, and abundance of Pacific cod, *Gadus macrocephalus*, in the Bering Sea. J Ichthyol 35: 18-27.

Stierhoff KL, Targett TE, Power JH (2009) Hypoxia-induced growth limitation of juvenile fishes in an estuarine nursery: assessment of small-scale temporal dynamics using RNA:DNA. Can J Fish Aquat Sci 66: 1033-1047.

Suryan RM, Irons DB, Brown ED, Jodice PGR, Roby DD (2006) Site-specific effects on productivity of an upper trophic-level marine predator: Bottom-up, top-down, and mismatch effects on reproduction in a colonial seabird. Prog Oceanogr 68: 303-328.

Tanasichuk RW, Kristofferson AH, Gillman DV (1993) Comparison of some life history characteristics of Pacific herring (*Clupea pallasi*) from the Canadian Pacific Ocean and Beaufort Sea. Can J Fish Aquat Sci 50: 964-971.

Tanasichuk RW, Ware DM, Shaw W, McFarlane GM (1991) Variations in diet, daily ration, and feeding periodicity of Pacific hake (*Merluccius productus*) and spiny dogfish (*Squalus acanthias*) off the lower west coast of Vancouver Island. Can J Fish Aquat Sci 48: 2118-2128.

Tanimoto Y, Iwasaka N, Hanawa K, Toba Y (1993) Characteristic variations of sea surface temperature with multiple time scales in the North Pacific. J Clim 6: 1153-1160.

Theilacker GH, Bailey KM, Canino MF, Porter SM (1996) Variations in larval walleye pollock feeding and condition: A synthesis. Fish Oceanogr 5 (Suppl 1): 112-123.

Thuiller W (2007) Climate change and the ecologist. Nature 448: 550-552.

Tong XH, Liu QH, Xu SH, Li J, Xiao ZZ, Ma DY (2010) Changes in RNA, DNA, protein contents and growth of turbot *Scophthalmus maximus* larvae and juveniles. J Fish Biol 77: 512-525.

Treberg JR, Hall JR, Driedzic WR (2005) Enhanced protein synthetic capacity in Atlantic cod (*Gadus morhua*) is associated with temperature-induced compensatory growth. Am J Physiol-Reg Integr Comp Physiol 288: R205-R211.

Trenberth KE (1990) Recent observed interdecadal climate changes in the Northern Hemisphere. Bull Am Meteorol Soc 71: 988-993.

Venrick EL, McGowan JA, Cayan DR, Hayward TL (1987) Climate and chlorophyll-a: long-term trends in the central North Pacific Ocean. Science 238: 70-72.

Vollenweider JJ, Heintz RA, Schaufler L, Bradshaw R (2011) Seasonal cycles in whole-body proximate composition and energy content of forage fish vary with water depth. Mar Biol 158: 413-427.

Walther G-R, Post E, Convey P, Menzel A, Parmesank C, Beebee TJC, Fromentin J-M, Hoegh-Guldberg O, Bairlein F (2002) Ecological responses to recent climate change. Nature 416: 389-395

Weber LP, Higgins PS, Carlson RI, Janz DM (2003) Development and validation of methods for measuring multiple biochemical indices of condition in juvenile fishes. J Fish Biol 63: 637-658.

Wespestad VG, Fritz LW, Ingraham WJ, Megrey BA (2000) On relationships between cannibalism, climate variability, physical transport, and recruitment success of Bering Sea walleye pollock (*Theragra chalcogramma*). ICES J Mar Sci 57: 272-278.

Williams EE, Somero GN (1996) Seasonal-, tidal-cycle-, and microhabitat-related variation in membrane order of phospholipid vesicles from gills of the intertidal mussel *Mytilus californianus*. J Exp Biol 199: 1587-1596.

Womble JN, Willson MF, Sigler MF, Kelly BP, VanBlaricom GR (2005) Distribution of Steller sea lions *Eumetopias jubatus* in relation to spring-spawning fish in SE Alaska. Mar Ecol Prog Ser 294: 271-282.

Zhang CI (1984) Pacific cod of South Korean waters. Bull Int N Pac Fish Comm 42: 116-129.

Appendix

To: Chair, UAF Institutional Animal Care and Use Committee, PO Box 757270, Fairbanks, Alaska 99775

Thru: WW Smoker, Advisory Committee Chair, Ashwin Sreenivasan, SFOS, 17101 Pt Lena Loop Rd, Juneau, Alaska 99801

From: SD Rice, Program Manager of Habitat and Marine Chemistry, US NOAA Fisheries, Alaska Fisheries Science Center, Juneau, Alaska

Subj: Animal Care and Use in Ashwin Sreenivasan's Dissertation Research

Ashwin Sreenivasan, a PhD Candidate at UAF in Fisheries, has been conducting his dissertation research in our laboratory; he has measured nucleic acid ratios in tissue samples as indexes of growth of individual fish.

In all cases, all tissue samples were supplied to Ashwin from our experiments, by federal scientists from our laboratory. Ashwin was not responsible for the collection, care, or handling of the fish sampled in this project; all animal collections, care, and handling were the responsibility of our federal scientists, and complied with the internal standards of our agency. We conducted a series of measurements; the tissue samples supplied to Ashwin were an additional measurement, and we did not alter our experimental designs and sampling procedures to accommodate the additional measurements, but were indeed opportunistic samples. The additional measurements by Ashwin were a valuable contribution to the study; he was responsible for the biochemical analyses of these tissue samples and the interpretation of these analyses.